Nos. 14-1139, 14-1142, and 14-1144

IN THE UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

ARIOSA DIAGNOSTICS, INC., NATERA, INC., and VERINATA HEALTH, INC.,
Plaintiffs-Appellees,
and
DNA DIAGNOSTICS CENTER, INC.,
Counterclaim Defendant-Appellee
and
THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY,
Plaintiff,
v.
SEQUENOM, INC. and SEQUENOM CENTER FOR MOLECULAR MEDICINE, LLC
Defendants-Appellants
and
ISIS INNOVATION LIMITED,
Defendant.

Appeals from the United States District Court for the Northern District of California, Judge Susan Illston

CONSOLIDATED OPENING BRIEF OF APPELLANT SEQUENOM, INC.

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January 21, 2014

CERTIFICATE OF INTEREST

Pursuant to Federal Circuit Rule 47.4, undersigned counsel for Appellant Sequenom, Inc. certifies the following:

1. The full name of every party represented by me in this appeal is: Sequenom, Inc.

2. The names of the real parties in interest, if different from the parties named above, are: Not applicable.

3. The names of all parent corporations and any publicly held companies that own 10% or more of the stock of the party represented by me are: None.

4. The names of all law firms and the partners or associates that appeared for Appellant in the district court or are expected to appear in this court are:

> Kaye Scholer LLP: Michael J. Malecek, Peter E. Root, Stephen C. Holmes, Aton Arbisser, Gary Ross Allen, Hanna Cohen, Robert Estrin, and Lily Robinton.

Dated: January 21, 2014

/s/ Michael J. Malecek
Michael J. Malecek
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STATEMENT OF RELATED CASES

There has been one previous appeal in these consolidated cases. In *Aria Diagnostics, Inc. v. Sequenom, Inc.*, Case No. 12-1531, 726 F.3d 1296 (Fed. Cir. 2013), decided on August 9, 2013, a panel of Chief Judge Rader and Judges Dyk and Reyna vacated and remanded on several grounds the District Court's order denying Sequenom's motion for a preliminary injunction, including reversing the District Court's claim construction and findings on equitable issues. The panel also remanded to the District Court for further consideration whether U.S. Patent No. 6,258,540 ("the '540 patent") satisfies 35 U.S.C. § 101, the issue again before this Court. *Id*. at 1304.

Federal Circuit Internal Operating Procedure # 3 applies to this appeal.

STATEMENT OF JURISDICTION

The District Court entered final judgments in two of the appealed cases and final judgment on the ’540 patent in the third case. This Court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(1).

STATEMENT OF THE ISSUES

A. Whether the claims of the ‘540 patent recite patent-eligible subject matter under 35 U.S.C. §101 where (1) the patent does not claim a natural phenomenon, but instead claims only a limited method that makes use of and applies the natural phenomenon, and (2) the claimed method does not preempt all uses of the natural phenomenon.

B. Whether the District Court committed reversible error when, in considering the issue of preemption, it refused to consider alternative methods of using the natural phenomenon unless such methods were both (1) first disclosed before the challenged patent was filed and (2) proven to be “commercially viable.”

C. Whether the District Court committed reversible error when it separated each step of the patented method and determined if each individual step, standing alone, was “inventive” for purposes of Section 101.

STATEMENT OF THE CASES AND FACTS

A. The Inventors Made The Pioneering Invention To Detect Paternally-Inherited Cell-Free Fetal DNA In Maternal Blood Products That Had Previously Been Discarded As Waste.

For decades before 1996, medical professionals and scientists had analyzed fetal DNA for prenatal diagnosis by relying only on invasive methods, such as amniocentesis and chorionic villus sampling ("CVS"), which "presented risks to the fetus and the mother." *See Aria*, 726 F.3d at 1299 (summarizing the factual and scientific background of the '540 patent). Researchers had also sought to isolate fetal DNA through alternative methods that focused on extracting intact fetal cells which passed into maternal blood through the amniotic sac during pregnancy. *Id*. In this work, researchers routinely discarded the cell-free fractions of maternal blood, including the plasma and serum. *Id*.

In an original stroke of genius, in 1996, Dr. Dennis Lo and Dr. James Wainscoat discovered cell-free fetal DNA ("cffDNA") in maternal plasma and serum — that portion of maternal blood samples that other researchers had previously discarded as medical waste. *Id*.; Joint Appendix ("A") 0183, ¶¶ 20-21. Drs. Lo and Wainscoat described their landmark discovery of cffDNA in maternal plasma and serum in a Lancet article which has been cited over a thousand times.

Lo and Wainscoat used the knowledge gained from their discovery to invent a specific, limited method to detect and analyze fetal DNA, and, through this

method, created "a paradigm shift in non-invasive prenatal diagnosis." A0196, ¶ 52; A0188-0192, ¶¶ 33-41. Applying a combination of laboratory techniques to their discovery, Lo and Wainscoat invented a method for detecting paternally-inherited cffDNA to determine fetal characteristics, such as gender, RhD status, and chromosomal aneuploidies. *See Aria*, 726 F.3d at 1299; A0039-0040, 2:61-3:62.

The method invented by Lo and Wainscoat solved the particular problem that cell-free fetal DNA is largely indistinguishable in maternal blood from cell-free maternal DNA. A0038, 2:57-59. Their invention focused on detection of the small fraction of cffDNA in the mother's plasma or serum the fetus had inherited from the father — as little as 0.13 percent of the DNA in the sample —and then further focused on the even smaller fraction of paternally-inherited sequences that were not also possessed by the mother. *Id.*; A0352.

This pioneering invention, as commercialized by Sequenom in its MateriT21 test, has created an alternative for prenatal diagnosis of fetal DNA that avoids the risks to the fetus and the mother inherent in widely-used techniques like amniocentesis and CVS. *Aria*, 726 F.3d at 1299; A0158-0159, ¶¶ 10-11.

B. The '540 Patent Recites A Limited Method To Detect Paternally-Inherited Cell-Free Fetal DNA.

"[T]he '540 patent claims methods to detect fetal genetic characteristics by analyzing cffDNA obtained from a maternal blood sample." *Aria*, 726 F.3d at

1299. The method enables the detection of paternally-inherited sequences within cffDNA that differ from the mother's own DNA sequences. *Id.* at 1301.

The '540 patent involves a three-step combination:

(1) Fractionating maternal blood to produce plasma or serum samples; *see Aria*, 726 F.3d at 1299.

(2) Amplifying paternally-inherited fetal nucleic acid from the samples, *id.* at 1303 (discussing construction of "amplifying"); and

(3) Detecting paternally-inherited fetal nucleic acid in the samples. *Id.* at 1301-02 (discussing construction of "paternally-inherited").

See Addendum 5 (Claims 1, 21, 24, and 25). Dependent claims further limit this method to specified, bounded uses. *See, e.g.*, Claim 5 (limiting method to fetal nucleic acid sequence on Y-chromosome), Claim 8 (limiting method to fetal nucleic acid from paternally-inherited non-Y-chromosome), Claims 19 and 20 (limiting method to fractional concentrations), Claim 23 (limiting method to clotting in maternal samples).[1]

[1] *See also* claims 6 (limiting method to fetal nucleic acid sequence on the DSY14 locus of Y-chromosome), 7 (limiting method to fetal nucleic acid sequence on the SRY gene of Y-chromosome), 12 (limiting method to determining sex of the fetus), 13 (limiting method to determining concentration of the fetal nucleic acid sequence in the maternal serum or plasma), 15 (limiting method to detecting a fetal or maternal condition in which the level of fetal DNA in the serum or plasma is higher or lower than normal), and 18 (limiting method to detection of a fetal chromosomal aneuploidy). The District Court did not address these six additional

(continued...)

Before the invention of Lo and Wainscoat, no one had applied the techniques of fractionating a pregnant woman's blood to create a plasma or serum cell free sample, amplifying the DNA in that sample, and detecting the specific paternally-inherited nucleic acids present in that sample. Nor had anyone *applied these techniques in combination* to characterize fetal genomic makeup to provide a safe alternative to the conventional invasive methods for analyzing fetal DNA in pregnant women. A0142, 1:11-2:5; A0183, ¶¶ 20-21; A0191-0192, ¶¶ 38-41, A0197-0201, ¶¶ 57-72.

C. The '540 Patent's Method Transforms Naturally-Occurring cffDNA To Detect Fetal Characteristics From Paternally-Inherited cffDNA.

The patented three-step method transforms cffDNA from its naturally-occurring state.

The patent's "fractionating" step involves separating plasma and serum from whole blood collected from a pregnant woman. The laboratory technician centrifuges tubes of whole blood with an anticoagulant, separating out the liquid plasma portion. A0039-0040, 2:19-21, 26-27; 4:26-27, 38-51. Serum is the product left after the technician then removes the clotting factors from the plasma. A0194, ¶ 44. This plasma and serum are what was previously discarded as ***waste***

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dependent claims. The parties stipulated that, if the District Court's summary judgment ruling is not reversed, these claims also fall under the District Court's reasoning. Addendum 3, 4.

by researchers looking for fetal DNA in intact fetal cells. *Aria*, 726 F.3d at 1299. As the patent recites, the laboratory technician pipettes the supernatant plasma and serum into fresh tubes separate from the blood-cell-containing "buffy coat," and then subjects the plasma and serum samples to a "second centrifugation." A0040, 4:37-51.

In the "amplifying" step of the patent, DNA is extracted from the fractionated serum or plasma samples, and is amplified by PCR or another method. "PCR is a biochemical technique that enables measurement of relatively small quantities of nucleic acids by iteratively and exponentially 'amplifying' a sample to detectable levels." *Board of Trustees of the Leland Stanford Junior University v. Roche Molecular Systems*, 583 F.3d 832, 837 (Fed. Cir. 2009) (reversing invalidity finding on method using PCR to detect quantity of HIV cells in blood).

As used in the invention, amplification by PCR requires heating DNA isolated from the serum or plasma sample to high temperatures to "denature" the originally double-stranded DNA into two single-stranded DNA pieces by melting the hydrogen bonds between complementary nucleotides. After cooling, the laboratory technician adds nucleotide bases, and synthetic primers that anneal to the 3' ends of the two single-stranded fragments, introduces Taq polymerase enzymes, and induces numerous TaqMan amplification reactions. A0042, 7:4-30; A0192, ¶ 42; *see generally Hoffman-La Roche, Inc. v. Promega Corp.*, 323 F.3d

1354, 1358 (Fed. Cir. 2003) (describing PCR process). The primers anneal to portions of the separated single-stranded fragments, which serve as templates for synthesizing new DNA strands. DNA polymerase enzymes extend the primers to fill in the intermediating sequence in the template strands, synthesizing two identical double-stranded DNA fragments from the separated single strands of the original helix. A0192, ¶ 42; *Promega,* 323 F.3d at 1358.

This process of strand separation, primer annealment, and extension is repeated over numerous cycles, producing exponential amounts of double-stranded DNA segments. A thermocycler alters the reaction temperature frequently to promote DNA denaturing and synthesis. A0042, 7:26-30; *see also* Sequenom's Request for Judicial Notice ("RJN") at 12-13, and accompanying Declaration of Michael J. Malecek ("Malecek Dec."), ¶ 7 & Exh. F. Amplification of cffDNA as achieved by PCR does not occur in nature — cffDNA exists outside of the cell, by definition, and thus, does not naturally replicate, requiring the synthetic creation of new DNA copies using building blocks provided by laboratory scientists. *See* RJN at 9-10, Malecek Dec., ¶ 5 & Exh. D (DNA synthesis/replication occurs only during the S phase of the cell cycle, within the nucleus of a cell).

Naturally-occurring cffDNA is transformed by PCR amplification. First, PCR products are *physically different* from naturally-occurring cell-free fetal DNA. The synthetic primers used in PCR attach to complementary target

sequences and, when two primers are used in a head-to-head orientation, the target sequences and the DNA sequences between those targets are amplified. If "universal" or "linker-primed" PCR is used, this produces a longer segment than the natural cffDNA, but of a fixed length with a specific sequence. *See* RJN at 12-14, Malecek Dec., ¶ 7 & Exh. F. Other PCR processes generally produce shorter products than the naturally-occurring cffDNA template. *Id.*; A0192-0193, ¶ 42.

Second, PCR products are *chemically different* from naturally-occurring cffDNA. In vertebrate DNAs, a large fraction of CpG sites are methylated; fetal DNA is highly methylated. *See* RJN at 9-11, Malecek Dec., ¶¶ 5-6 & Exhs. D, E. Methylation occurs when enzymes within the cell take a methyl group and transfer the group to the 5 position of the base cytosine (C) when it is followed in the DNA sequence by the base guanine (G) ("a CpG site"). *See* RJN at 11, Malecek Dec., ¶ 5 & Exh. D. Compared to naturally-occurring cffDNA, copies of fetal DNA created in a laboratory through amplification lack methyl groups chemically bound to the CpG sites, differentiating them chemically. *See* RJN at 12, 14, Malecek Dec., ¶ 8 & Exh. G.

The "detecting" step of the claimed method requires additional laboratory manipulation. The lab technician adds the amplified DNA to an agarose gel containing ethidium bromide to stain and visualize the DNA. A0041, 5:23-24. Alternatively, DNA polymerase cleaves inserted probes (i.e., short

oligonucleotides) with fluorescent reporter dyes attached while synthesizing the complementary nucleotides strand. As the patent recites, a "real time sequence detector is able to measure the fluorescence intensity of the liberated reporter molecules cycle after cycle. . . . An amplification reaction in which the fluorescence intensity rises above the threshold during the course of thermal cycling is defined as a positive reaction." A0041, 6:36-59; A0043, 10:12-21.

D. The '540 Patent's Method Is Only One Among Several Alternative Methods Using cffDNA.

The claimed methods recited in the '540 patent are not the only methods for detecting fetal DNA (including cffDNA). Several alternative practical methods have been used to make prenatal diagnoses from cffDNA without duplicating the '540 patent's method. These alternatives do not include at least one of the '540 patent's essential limiting steps. Thus, there are peer-reviewed, scientifically validated methods that do not require fractionation, or do not require amplification, or do not detect paternally-inherited cffDNA. The existence of these alternative methods demonstrates that the '540 patent does not preempt all uses of cffDNA. The District Court, however, refused to consider these alternatives in finding, incorrectly, that the '540 patent preempts all uses of a natural phenomenon. Opinion at 19.

1. Methods Without Fractionation.

Researchers, such as Ariosa's expert Dr. Farideh Bischoff, champion a method that detects cffDNA from whole maternal blood without removing the cellular component — that is, without fractionating the maternal plasma or serum as the '540 patent requires. A0338-0340; A0229-0230, ¶ 27. Dr. Bischoff's team "devised a reliable method of fetal DNA detection using dried maternal blood specimens." A0338.

Dr. Bischoff's results from "whole blood samples" showed "Y-chromosome specific sequences were detected in all 19 (100%) pregnancies confirmed to have a male fetus." A0339. This "simple method . . . enabl[es] cell-free fetal DNA to be incorporated into non-invasive screening regimes." A0340.

2. Methods Without Amplification.

Other researchers detect aneuploidies in cffDNA using methods not involving amplification, much less amplification of paternally-inherited cffDNA as the '540 patent requires. A0342-0349. The van den Oever team accurately detected eleven trisomy-21 cases. A0345-0346. "[I]n this study, we have demonstrated successful fetal T21 detection using free DNA from maternal plasma by single molecule sequencing on the Helicos platform." A0348. Single molecule sequencing involves no DNA amplification. *Id*.

3. Methods Without Paternally-Inherited cffDNA.

Yet another method locates fetal markers in cffDNA without distinguishing between paternally-inherited and maternally-inherited DNA. A0359-0365. Poon and his colleagues detected cffDNA in maternal plasma from methylated alleles without identifying paternally-inherited cffDNA. A0360. They found that "it is possible to detect a maternally inherited fetal allele from maternal plasma." A0364. In contrast, the '540 patent's method is expressly limited to detecting paternally-inherited nucleic acids in plasma or serum.

E. The District Court Invalidated The '540 Patent For Claiming Patent-Ineligible Subject Matter Under Section 101.

In December 2011, Ariosa Diagnostics, Inc. filed a declaratory judgment action alleging it does not infringe the '540 patent, of which Sequenom is the exclusive licensee. A0058, docket no. 1. Sequenom counterclaimed for infringement. A0061, docket no. 33. In early 2012, Natera, Inc. and Verinata Health, Inc., two other competitors of Sequenom, each brought similar actions, and Sequenom counterclaimed. A0093, docket no. 1; A0096, docket no. 40; A0115, docket no. 1; A0116, docket no. 15. The District Court related the three actions for pretrial purposes. A0062, docket no. 41.[2]

[2] Verinata's action also alleges that Sequenom infringed certain of Verinata's patents. Those patents are not at issue in these appeals.

In July 2012, the District Court denied Sequenom's preliminary injunction motion, finding, in part, a substantial question whether the '540 patent's claims are eligible under Section 101. *See Aria*, 726 F.3d at 1304. This Court reversed:

> Because the district court did not have the benefit of [*Ass'n for Molecular Pathology v. Myriad Genetics, Inc.*, 133 S. Ct. 2107 (2013)] and also in light of this court's disagreement with the district court's claim construction, this court remands for the district court to examine subject matter eligibility in the first instance.

Id. at 1304.

After remand and claim construction, Ariosa sought summary judgment as to subject matter eligibility, and Sequenom cross-moved. On October 30, 2013, the District Court entered summary judgment for Ariosa. *See* Addendum 1 (reported at __ F. Supp.2d __, 2013 WL 586022 (N.D. Cal. 2013)).

In its Opinion, the District Court determined that the presence of cffDNA in the blood of a pregnant woman is a natural phenomenon. Opinion at 12. The District Court acknowledged that "the '540 patent does not claim as an invention the discovery of cffDNA in maternal plasma or serum." *Id*. Rather, "[t]he '540 patent claims methods of detecting paternally inherited cffDNA in maternal plasma or serum." *Id*.

The District Court divided the claims into individual steps and considered each technique in each step separately, as opposed to examining the claimed method as a whole. *Id*. at 13. The District Court did not determine whether the

combination of steps constituting the entire claimed process had been used previously, but instead found that, when the patent was filed, each step's laboratory technique, considered separately, was "well-understood, routine, and conventional activity." *Id.* at 13-15. Based on this conclusion, the District Court held that "the method steps contained in claims 1, 2, 4, 5, 8, 19-22, 24, and 25 of the '540 patent do not add enough to the natural phenomenon of paternally inherited DNA to make these claims patentable under §101." *Id*. at 13.

The District Court rejected Sequenom's argument that its method is a patent-eligible use or application of cffDNA. *Id.* at 15. The District Court concluded:

> It is only an innovative or inventive use of a natural phenomenon that is afforded patent protection . . . But, based on the undisputed facts before the Court, the only inventive part of the patent is that the conventional techniques of DNA detection known at the time of the invention are applied to paternally inherited cffDNA as opposed to other types of DNA. Thus, the only inventive concept contained in the patent is the discovery of cffDNA, which is not patentable.

Id. The District Court further found that, "looking at the claimed processes as a whole, the only inventive component of the processes in the '540 patent is to apply those well-understood, routine processes to paternally inherited cffDNA, a natural phenomenon." *Id*. at 18.

Finally, the District Court stated that "a court should consider whether the claim poses a risk of preempting a law of nature, natural phenomenon, or abstract idea." *Id*. It refused, however, to give any significance to Sequenom's evidence of

three alternative peer-reviewed, non-infringing methods of using cffDNA in maternal blood. *Id*. at 19. The District Court held that evidence of alternatives showing non-preemption is relevant *only* when the alternative methods were both (i) publicly disclosed before the challenged patent was filed and (ii) shown to be "commercially viable." *Id.*

The District Court found that "the articles cited by Sequenom were published after the issuance of the patent and well after the date of the invention." *Id*. The District Court also determined that no alternative method was commercially viable because "twelve years have passed since the issuance of the patent but Sequenom does not present the Court with any evidence of a commercially viable alternative method of detecting cffDNA." *Id*. Relying on these conclusions, the District Court rejected Sequenom's evidence of alternative non-preemptive methods, and concluded that, on the evidence it had considered, "it appears that the effect of issuing the '540 patent was to wholly preempt all known methods of detecting cffDNA at that time." *Id.*

The parties stipulated to final judgments on the '540 patent issues in all three cases based on the District Court's summary judgment opinion. *See* Addendum at 2-4. This Court consolidated the three appeals.

SUMMARY OF ARGUMENTS

The District Court misapplied Supreme Court and Federal Circuit law. This Court should reverse.

The '540 patent does not claim a natural phenomenon or a naturally occurring process. Nor does it preempt the use of fetal DNA, and specifically cffDNA. Rather, it claims a specific, non-preemptive, and limited diagnostic method using fetal DNA found in cell-free form in the serum or plasma in maternal blood. This method transforms naturally-occurring cffDNA by a three-step process of fractionation, amplification, and detection of paternally-inherited sequences.

Whether a patent applying a natural phenomenon preempts all other uses of the phenomenon is a primary principle motivating the judicial exceptions to Section 101. In considering the issue of preemption, the District Court erred by discounting entirely Sequenom's evidence of other, scientifically-validated alternative methods using cffDNA.

The undisputed evidence before the District Court is that there are several practical, peer-reviewed, non-infringing alternative methods to detect and use cffDNA which were invented since the '540 patent issued. Patent law encourages this innovation through burgeoning future uses of a natural phenomenon. Section 101's preemption doctrine allows and protects all of these applications, including

the first-disclosed application which, as evidenced by the existence of later alternative methods, could not have been preemptive. Had the District Court properly considered this evidence rather than imposing a new two-part standard of its own to dismiss the evidence, it could not have invalidated the patent under Section 101.

The District Court adopted an unprecedented and improper standard to determine the relevance of alternative methods offered to prove the claimed method does not preempt all uses of a natural phenomenon. It held that an alternative method would be relevant to show lack of preemption only if it *both* (i) was disclosed publicly before the '540 patent issued, *and* (ii) was shown by Sequenom to be commercially viable. Neither Supreme Court nor Federal Circuit authority supports the standard adopted by the District Court.

The requirement that alternative methods have been "previously disclosed" defies logic, especially for ground-breaking inventions. Inventors like Lo and Wainscoat would be unable to patent their method for using their discovery until others had already come up with and publicly disclosed their own alternative methods. Inventors would hold back on developing or applying to patent new methods, defeating the incentive to innovate and disclose that underlies all patent law.

The requirement that alternative methods be shown by the patent owner to be "commercially viable" has never been approved by any other court. It would irrationally exclude alternative methods that are patent eligible under Section 101, which requires only that such methods be useful, not commercially viable. The District Court's analysis also frustrates innovation by invalidating those patents that teach the best, and thus likely the most commercially successful, methods of applying natural phenomena.

The method of the '540 patent involves a three-step process of fractionation, amplification, and detection of paternally-inherited sequences. Other methods which omit the fractionation or amplification steps, or which detect nucleotides without regard to their maternal or paternal source, fall outside the '540 patent. The undisputed evidence is that there are several practical, peer-reviewed, non-infringing alternative methods to detect and use cffDNA. Had the District Court considered this evidence, the District Court could not have invalidated the patent under Section 101.

This Court should conclude that the claims of the '540 patent are non-preemptive and are drawn to patent-eligible subject matter. For this reason alone, this Court should reverse the District Court's judgments.

The District Court also misapprehended the Supreme Court's direction that Section 101 requires an "inventive concept." Contrary to the District Court's view,

the "inventive concept" requirement does not require that the individual elements of a claim, considered separately and apart from the natural phenomenon, must be novel or non-conventional to be patent-eligible under Section 101. Instead, Supreme Court precedent requires only that the combination of elements must, in practice, amount to more than a claim to the natural phenomenon itself.

The '540 patent meets this requirement. According to the invention, the maternal plasma or serum must be fractionated from the whole blood, the paternally-inherited cffDNA must be amplified, and thus transformed, by laboratory techniques to produce detectable quantities, and a means of detecting the nucleic acids—such as with fluorescent labels or other dyes—must be introduced to enable detection. The claims of the '540 patent are meaningfully limited, and thus contain the requisite inventive concept.

Further, the District Court disregarded the undisputed fact that no one had ever before combined fractionation, amplification, and detection protocols into a method of identifying paternally-inherited cffDNA in maternal serum or plasma for use in diagnosing fetal characteristics. Instead of considering the combined patented method as a whole in accordance with Supreme Court precedent, the District Court improperly dissected the claim elements and considered each step independently. The inventors of the '540 patent applied their discovery of cffDNA

in maternal plasma and serum to a new and useful end. Their claimed method is patent-eligible.

Finally, the District Court's decision misapplies *Association for Molecular Pathology v. Myriad Genetics*, 133 S. Ct. 2107 (2013). Previously, this Court directed the District Court to re-consider patent eligibility "in light of" *Myriad*. *Myriad* provided three points of certainty about what the Supreme Court accepts as patent-eligible.

First, a patent on a natural phenomenon or law of nature itself, such as the nucleotide sequence of the BRCA genes, fails Section 101. The '540 patent does not claim cffDNA in maternal blood.

Second, a patent that transforms a naturally-occurring phenomenon into matter not found in nature, such as cDNA, satisfies Section 101, even for composition claims available for any use whatsoever. Because laboratory-amplified nucleic acids differ chemically and physically from naturally-occurring cffDNA in maternal blood, the '540 patent falls outside the Section 101 exception. Moreover, unlike the *Myriad* composition patents, the '540 patent is limited to bounded method claims.

Third, a method combining known laboratory techniques into a new and useful method of using a discovery is also patent-eligible, as exemplified by the Supreme Court's view on Myriad's Claim 21. Like Claim 21, the '540 patent's

claims combine known laboratory techniques in a method and apply that method to a new discovery for a "new and useful end."

For any or all of these reasons, this Court should reverse and remand.

ARGUMENT

I. THIS COURT REVIEWS THE DISTRICT COURT'S DECISION DE NOVO, CONSTRUES SECTION 101 EXPANSIVELY, AND APPLIES JUDICIAL EXCEPTIONS NARROWLY.

A. This Court Reviews The Section 101 Issue De Novo.

This Court reviews a grant of summary judgment under the regional circuit's law. *See Accenture Global Services v. Guidewire Software, Inc.*, 728 F.3d 1336, 1340 (Fed. Cir. 2013). The Ninth Circuit reviews summary judgments *de novo*. *See Heinemann v. Satterberg*, 731 F.3d 914, 916 (9th Cir. 2013). This Court applies its "own law, however, with respect to issues of substantive patent law." *CLS Bank Int'l v. Alice Corp. Pty. Ltd.*, 717 F.3d 1269, 1276 (Fed. Cir. 2013) (en banc), *cert. granted*, 82 U.S.L.W. 3346 (U.S. Dec. 6, 2013) (No. 13-298).

"Patent eligibility under § 101 presents an issue of law that we review *de novo*." *Id. See Ultramercial, Inc. v. Hulu, LLC*, 722 F.3d 1335, 1338 (Fed. Cir. 2013) ("This court also reviews the ultimate determination regarding patent-eligible subject matter under 35 U.S.C. § 101 without deference."), *cert. filed*, 82 U.S.L.W. 3107 (U.S. Aug. 23, 2013) (No. 13-255).

B. Section 101 Must Be Construed Expansively And Its Exceptions Must Be Applied Narrowly.

"In cases of statutory construction, we begin with the language of the statute." *Diamond v. Diehr*, 450 U.S. 175, 182 (1981). "The statute controls the inquiry into patentable subject matter." *Ultramercial*, 722 F.3d at 1340. Section 101 of the Patent Act provides:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

35 U.S.C. § 101.

The wide sweep of what is patent-eligible reflects Congressional intent. "In choosing such expansive terms . . . modified by the comprehensive 'any,' Congress plainly contemplated that the patent laws would be given wide scope." *Bilski v. Kappos*, 130 S. Ct. 3218, 3225 (2010). "As the Supreme Court has explained, Congress intended that the statutory categories would be broad and inclusive to best serve the patent system's constitutional objective of encouraging innovation." *CLS*, 717 F.3d at 1276. *See also id*. ("[T]he categories of patent-eligible subject matter recited in § 101 are broad . . . ."); *Ultramercial*, 722 F.3d at 1341 ("At a time when Congress considered § 101, it broadened the statute and certainly did not place any specific limits on it.").

The judicially-created exceptions to Section 101 — barring patents claiming natural phenomena, laws of nature, and abstract ideas — must be applied "narrowly." *Bilski*,130 S. Ct. at 3229. *See also CLS*, 717 F.3d at 1277 ("[D]anger also lies in applying the judicial exceptions too aggressively because 'all inventions at some level embody, use, reflect, rest upon, or apply laws of nature, natural phenomena, or abstract ideas.'" (quoting *Mayo Collaborative Servs. v. Prometheus Labs., Inc.*, 132 S. Ct. 1289, 1293 (2012)). As this Court has explained:

> To sum up, because eligibility requires assessing judicially recognized exceptions against a broad and deliberately expanded statutory grant, one of the principles that must guide our inquiry is these exceptions should apply narrowly. Indeed, the Supreme Court has cautioned that, to avoid improper restraints on statutory language, acknowledged exceptions thereto must be rare.

Ultramercial, 722 F.3d at 1342.

Section 101 provides a "threshold test" of eligibility, *Bilski*, 130 S. Ct. at 3225, not a test of substantive validity. *See CLS*, 717 F.3d at 1276 ("Congress's broad approach to subject-matter eligibility ensures that the patent office doors remain open to most inventions"). Thus, "to override the broad statutory categories of eligible subject matter," the "disqualifying characteristic" of an exception to Section 101 must exhibit itself "manifestly." *Research Corp. Techs., Inc. v. Microsoft Corp.*, 627 F.3d 859, 868 (Fed. Cir. 2010). "Taken too far, the exceptions could swallow patent law entirely." *CLS*, 717 F.3d at 1277.

C. Only Clear And Convincing Evidence Can Rebut The '540 Patent's Presumption Of Eligibility Under Section 101.

The general presumption of patent validity applies fully to challenges under Section 101. *See CLS*, 717 F.3d at 1284 ("it bears remembering that all issued patent claims receive a statutory presumption of validity," and "that presumption applies when § 101 is raised as a basis for invalidity in district court proceedings"). The '540 patent is presumed to satisfy the eligibility requirements of Section 101.

Because of this presumption of validity, "any attack on an issued patent based on a challenge to the eligibility of the subject matter must be proven by clear and convincing evidence." *Ultramercial*, 722 F.3d at 1342. As Chief Judge Rader stated in his *CLS* opinion:

> Because we believe the presumption of validity applies to all challenges to patentability, including those under Section 101 and the exceptions thereto, we find that any attack on an issued patent based on a challenge to the eligibility of the subject matter must be proven by clear and convincing evidence. . . . We believe, moreover, that application of this presumption and its attendant evidentiary burden is consistent with the Supreme Court's admonition to cabin the judicially created exceptions to Section 101 . . . .

717 F.3d at 1304-05.

Clear and convincing evidence is evidence producing "an abiding conviction that the truths of [] factual contentions are 'highly probable.'" *Colorado v. New Mexico*, 467 U.S. 310, 316 (1984).

II. THE '540 PATENT DOES NOT CLAIM A NATURAL PHENOMENOM AND DOES NOT FALL WITHIN THE JUDICIALLY-CREATED NATURAL PHENOMENON EXCEPTION TO PATENT-ELIGIBILITY.

A. Controlling Precedent Establishes That A Method Applying A Natural Phenomenon Is Patent-Eligible Under Section 101.

Before the District Court, Ariosa did not attack the '540 patent for failing to satisfy the literal statutory requirements of Section 101. Ariosa's challenge, and the District Court's ruling, relied solely on the judicially-created "natural phenomenon exception" to Section 101 eligibility. Opinion at 5, 12.

As explained below, the '540 patent does not fall within the natural phenomenon exception. The '540 patent does not claim ownership of fetal DNA, the multiple forms of fetal DNA in maternal blood, cell-free fetal DNA in maternal blood, nor paternally-inherited DNA. Rather, it claims a specific, limited diagnostic method. This method does not claim ownership of a natural phenomenon, whether analyzed for "preemption," as discussed in Section B below, or "inventive concept," as discussed in Section C below

On the issue of preemption, Sequenom presented undisputed evidence describing three alternative practical, peer-reviewed, and non-preemptive ways of using cffDNA. The District Court's invalidation of the '540 patent based on this record was reversible error, resulting from its adoption of unprecedented and

improper requirements of commercial viability and predating the patent for considering proof of alternative, non-preemptive methods.

On the issue of inventive concept, Sequenom showed that the claimed method contains meaningful limitations. Further, to the extent more is required, the method reflects a significant human contribution in that Lo and Wainscoat combined and utilized man-made tools of biotechnology in a new way that revolutionized prenatal care. One simple measure of Lo and Wainscoat's contribution is that their 1997 Lancet publication has been cited over a thousand times. Future advancements in biotechnology are at significant risk if such an invention is found ineligible for patenting.

B. The Processes Claimed In The '540 Patent Do Not Preempt A Natural Phenomenon And Are Eligible Under Section 101.

1. Preemption Is A Primary Motivating Concern For Section 101 Eligibility Analysis.

The judicially-created exceptions to Section 101 rest on a core principle: patents cannot permissibly preclude all future uses of natural phenomena, natural laws, or abstract ideas. *See CLS*, 717 F.3d at 1277 ("The underlying concern is that patents covering such elemental concepts would reach too far and claim too much, on balance obstructing rather than catalyzing innovation."); *id*. at 1280 ("Preemption features prominently in the Supreme Court's recent § 101 decisions . . . .").

Therefore Congress cannot grant a legal monopoly over the exploitation of a natural phenomenon, law of nature, or abstract idea, all of which are "the handiwork of nature" and belong to everyone. *Funk Bros. Seed Co. v. Kalo Inoculant Co.*, 333 U.S. 127, 131 (1948). "Guarding against the wholesale preemption of fundamental principles should be our primary aim in applying the common law exceptions to § 101." *CLS*, 717 F.3d at 1281.

In *CLS*, this Court reviewed Supreme Court authority on Section 101 and concluded that the claims' preemptive effect on a fundamental concept is the primary determinant of Section 101 eligibility: "[T]he Supreme Court's foundational § 101 jurisprudence . . . turns primarily on the practical likelihood of a claim preempting a fundamental concept." 717 F.3d at 1277.

The *CLS* plurality opinion was categorical that preemption is the predominant Section 101 concern:

> First and foremost is an abiding concern that patents should not be allowed to preempt the fundamental tools of discovery. . . . Guarding against the wholesale preemption of fundamental principles should be our primary aim in applying the common law exceptions to § 101. . . . What matters is whether a claim threatens to subsume the full scope of a fundamental concept . . . .

Id. at 1280-81. Chief Judge Rader's concurring and dissenting opinion also reiterated that a patent-eligibility problem arises only "when a claim preempts all practical uses." *Id*. at 1300. *See also Accenture*, 728 F.3d at 1344 ("Having identified the abstract idea of the claim, we proceed with a preemption analysis to

determine whether . . . in practical terms, it does not so cover the full abstract idea itself.").

The District Court failed to respect the primacy and predominance of preemption in Section 101 analysis, concluding that preemption is only "a consideration when performing § 101 analysis." Opinion at 18 n.9. The District Court erred as a matter of law.

For over 150 years, the Supreme Court has reaffirmed that preemption principles lie at the heart of Section 101 law. *See Mayo,* 132 S. Ct. at 1301 ("The Court has repeatedly emphasized . . . a concern that patent law not inhibit further discovery by tying up the future use of laws of nature."). "What matters is whether a claim threatens to subsume the full scope of a fundamental concept, and when those concerns arise, we must look for meaningful limitations that prevent the claim as a whole from covering the concept's every practical application." *CLS*, 717 F.3d at 1281.

In *Ultramercial*, this Court described *O'Reilly v. Morse*, 56 U.S. 62 (1854), as "[a]n old example [but which] may be the most informative" on this point. 722 F.3d at 1344. *Morse* is the classic preemption case. *See Mayo*, 132 S. Ct. at 1301. Samuel Morse's first seven claims reciting methods applying his discovery of the telegraph were patent-eligible. However, Morse's eighth claim for "the use of the motive power of the electric or galvanic current" to communicate "intelligible

characters" "at any distances" was barred as patent-ineligible. *Morse*, 56 U.S. at 112-16. *See also Parker v. Flook*, 437 U.S. 584, 592 (1978) (*Morse* was a "landmark decision").

In contrast, in *The Telephone Cases*, 126 U.S. 1 (1888), the Supreme Court held that, because Alexander Graham Bell had claimed only methods "for transmitting vocal or other sounds telegraphically," his claims were all patent-eligible. *Id*. at 534-39. As the Supreme Court later explained, "Bell's claim, in other words, was not one for all telephone use of electricity." *Gottschalk v. Benson*, 409 U.S. 63, 69 (1972). "The concern underscoring *Morse*, which has become clearer through the Supreme Court's more recent precedents, is to deny patentability to an idea itself, rather than an application of that idea." *Ultramercial*, 722 F.3d at 1345.

The Supreme Court has variously stated the distinction it drew in the *Morse* and *Telephone* cases, but the essence is that a patent recites ineligible subject matter only when it claims for itself, or preempts all other uses of, an abstract idea, law of nature, or natural phenomenon. *See Bilski*, 130 S. Ct. at 3231 ("Allowing [the claims] would pre-empt use of this approach in all fields, and would effectively grant a monopoly over an abstract idea."); *Benson*, 409 U.S. at 71-72 ("The mathematical formula involved here has no substantial practical application except in connection with a digital computer, which means that if the judgment

below is affirmed, the patent would wholly pre-empt the mathematical formula and in practical effect would be a patent on the algorithm itself.").

Conversely, the concern about preemption of a natural law is not present when, as here, a patent *applies* a natural phenomenon in a limited, non-preemptive manner so that *other uses* may be made of it. *See Bilski,* 130 S. Ct. at 3230 ("[W]hile an abstract idea, law of nature, or mathematical formula could not be patented, an *application* of a law of nature or mathematical formula to a known structure or process may well be deserving of patent protection."); *Diehr,* 450 U.S. at 187 ("It is now commonplace that an application of a law of nature or mathematical formula to a known structure or process may well be deserving of patent protection."); *Intervet Inc. v. Merial Ltd.*, 617 F.3d 1282, 1293 (Fed. Cir. 2010) (Dyk, J., concurring and dissenting) (unlike isolated DNA sequence, "applications associated with the isolated nucleotide sequence . . . [may be] patentable subject matter").

As this Court recently explained, "It is not the breadth or narrowness of the abstract idea that is relevant, but whether the claim covers every practical application of that abstract idea." *Ultramercial,* 722 F.3d at 1346. If a patent implicates an identified abstract idea, law of nature, or natural phenomenon,

> [t]he §101 inquiry next proceeds to the requisite preemption analysis. With the pertinent abstract idea identified, the balance of the claim can be evaluated to determine whether it contains additional substantive limitations that narrow, confine, or otherwise tie down the

> claim so that, in practical terms, it does not cover the full abstract idea itself.

CLS, 717 F.3d at 1282. *See Accenture*, 728 F.3d at 1344-45 (stating same two-part preemption test).

Thus, a method applying or using a natural phenomenon in a manner that does not preclude alternative methods in the same field is non-preemptive, and, by definition, patent-eligible under Section 101. The '540 patent claims just such a method. The District Court's downgrading of preemption to merely "a consideration when performing a § 101 analysis" led it into reversible error.

The District Court also mistakenly characterized *Flook* and *Bilski* as cases invalidating a *non*-preemptive patent, and used this mischaracterization to devalue preemption as a Section 101 analytical tool. Opinion at 18 n.9. The District Court misread both cases.

In *Flook*, the patentee disclaimed use of his formula in some petrochemical-related functions, and argued his claim was therefore non-preemptive. *See* 437 U.S. at 589-90. The Supreme Court held that Flook's non-preemption argument "exalts form over substance." *Id*. at 590. A claim to the formula and nothing else, whether or not functionally self-limiting, is preemptive and fails Section 101. *See Accenture*, 728 F.3d at 1345 ("Accenture's attempts to limit the abstract concept to a computer implementation and to a specific industry thus do not provide

additional substantive limitations to avoid preempting the abstract idea of system claim 1.").

The District Court similarly mischaracterized *Bilski* as a non-preemption case. Opinion at 18 n.9. In *Bilski*, the patent claimed processes for hedging price risks in energy commodities markets. *See* 130 S. Ct. at 3223-34. The Supreme Court rejected the claims on preemption grounds: "Allowing petitioners to patent [the claims] would pre-empt use of this approach in all fields, and would effectively grant a monopoly over an abstract idea." *Bilski*, 130 S. Ct. at 3231.

The '540 patent does not claim exclusive use of cffDNA in maternal blood and the patent does not artificially self-limit their use, as the *Flook* and *Bilski* patentees sought to do with respect to the abstract idea or natural phenomenon at issue in those cases. Rather, the '540 patent claims one method of using cffDNA which is distinct from the several alternative methods available and which does not claim preemptive ownership over a natural phenomenon in any function or field.

2. The '540 Patent Does Not Claim A Natural Phenomenon And Does Not Preempt All Uses Of cffDNA.

The '540 patent neither claims a natural phenomenon nor claims a method that preempts a natural phenomenon. Instead, the '540 patent claims a method combining well-known laboratory techniques used for the first time to detect fetal characteristics from paternally-inherited fetal DNA from a particular sample type — cffDNA in maternal plasma or serum. The claimed method is but one among

several methods using and applying cffDNA from maternal blood. The District Court's decision that the '540 patent effectively claims all uses and applications of cffDNA in maternal blood, *see* Opinion at 19, misapplies the law and is contrary to the evidence.

In contrast to the method of the '540 patent, the alternative methods applying cffDNA either make use of whole blood rather than the fractionated plasma or serum, or do not amplify cffDNA, or search for fetal characteristics regardless of whether they are maternal or paternal in origin. *See* pages 9–11 *supra*. These differences set the '540 patent apart from, for example, the claims the Supreme Court invalidated in *Funk*, on which the District Court relied. Opinion at 6-7.

In *Funk*, the claim was for "a mixed culture of Rhizobia capable of inoculating the seeds of plants belonging to several cross-inoculation groups." 333 U.S. at 130. No specific combination of seeds was specified; the patent claimed *any* combination that worked. *Id*. The claim was to "no more than the discovery of some of the handiwork of nature and hence is not patentable." *Id*. at 131. As *Funk* explained, an invention can "come from the application of the law of nature to a new and useful end." *Id*. at 130. *See also Benson*, 409 U.S. at 67 ("If there is to be invention from such a discovery, it must come from the *application* of the

law of nature to a new and useful end.") (emphasis added). The '540 patent applies cffDNA to a new and useful end.

The '540 patent is analogous to the patent the Supreme Court validated in *Diehr*. The District Court inappropriately gave short shrift to *Diehr*. Opinion at 15-16.

Diehr recited a multi-step method for a molding process delivering rubber cured to the correct temperature and consistency. 450 U.S. at 177. The method determined the right time to open the mold by calculating the internal temperature through regular application of a mathematical formula, "the Arrhenius equation," an abstract idea. *Id.* at 177-79 & nn.2-5. Just as *Diehr* did not claim the Arrhenius equation but only one process applying the formula, *see id.* at 187, the '540 patent does not claim all uses of cffDNA in maternal blood but only one of several possible methods applying cffDNA. Combining steps in a new and useful method that is only one of several possible methods applying a fundamental concept, as in the '540 patent and the patent in *Diehr*, distinguishes those patents from the patent in *Flook*, on which the District Court substantially, and mistakenly, relied. Opinion at 8-9, 15-16, 18 n.9.

In *Flook*, the claim was for a formula to update alarm limits, an abstract idea, and nothing more. *See* 437 U.S. at 587-90. "The patent claims cover *any use* of respondent's formula for updating the value of an alarm limit on *any process*

variable involved in a process comprising the catalytic chemical conversion of hydrocarbons." 437 U.S. at 586 (emphasis added). *See Diehr*, 450 U.S. at 187 ("All that [the patent in *Flook*] provides is a formula for computing an updated alarm limit."); *Mayo*, 132 S. Ct. at 1299 ("And so the other steps [in the *Flook* patent] did not limit the claim to a particular application."). On the other hand, as with the '540 patent, *Diehr*'s claims were qualitatively different:

> In contrast [to *Flook*], the respondents here do not seek to patent a mathematical formula. Instead, they seek patent protection for a process for curing synthetic rubber. Their process admittedly employs a well-known mathematical equation, but they do not seek to pre-empt the use of that equation. Rather, they seek only to foreclose from others the use of that equation in conjunction with all of the other steps in their claimed process. . . . Arrhenius' equation is not patentable in isolation, but when a process for curing rubber is devised which incorporates in it a more efficient solution of the equation, that process is at the very least not barred at the threshold by § 101.

Diehr, 450 U.S. at 187-88.

Here, like *Diehr* and unlike *Flook*, the '540 patent "does not seek to pre-empt the use" of cffDNA in maternal blood. *Id*. at 187. Indeed, there are at least three scientifically-proven, non-infringing alternative methods that use cffDNA. "Because the applicant claimed a specific application, rather than an abstract idea in isolation, the claims satisfied § 101." *CLS*, 717 F.3d at 1279.

The District Court also misread the Supreme Court's decision in *Mayo*. Opinion at 12-13, 16. The District Court glossed over the primacy *Mayo* gave to preemption analysis.

The methods claimed in *Mayo* optimized administration of thiopurine based on a natural correlation between a dose's therapeutic efficacy and the concentration of thiopurine metabolites in the patient's blood. *See Mayo*, 132 S. Ct. at 1294-95. The Supreme Court held the patent was no more than a claim over the relationship between the dose level and metabolite concentration, a law of nature. *Id*. at 1298. The claimed "invention" did not change the process: a doctor administering thiopurine would have acted in exactly the same manner whether or not the patent was in effect, but, after the patent, the doctor's application of this prior art would have been an infringement. *Id*. The Supreme Court invalidated the *Mayo* patent to prevent it from preempting all uses of the natural correlation. *Id*.

As this Court explained, "the [Supreme] Court [in *Mayo*] held that those steps [in the patent's claimed method] failed to render the claims patent eligible because, as a practical matter, they were necessary to every practical use of what it found to be a natural law and therefore were not truly limiting." *CLS*, 717 F.3d at 1283. There could be no alternative non-preemptive method using the natural law in *Mayo*. This contrasts sharply with the '540 patent, whose claim limitations are "truly limiting" — as demonstrated by the several scientifically-validated, non-infringing alternatives using cffDNA.

In sum, the Supreme Court's and this Court's precedents follow a consistent theme: where claims recite a natural phenomenon and no more, or when they recite

a method in terms so general that it covers all ways to use the natural phenomenon, then the claims are not patent-eligible. Where, as here, the patent claims a specific limited method and there are alternative methods available, then there is no preemption and no Section 101 eligibility concern. The District Court failed to follow this controlling Section 101 law.

3. The District Court Wrongly Discounted Entirely Sequenom's Evidence Of Three Non-Preemptive Alternative Methods Using cffDNA.

Sequenom presented the District Court with evidence of three peer-approved, practical, alternative methods using cffDNA in maternal blood, none of which infringes the '540 patent. *See* 9-11 *supra*. The authenticity and veracity of Sequenom's evidence was undisputed.

These three peer-reviewed articles demonstrate that each of the three primary limitations of the '540 patent are truly "meaningful limitations." According to this evidence, as an alternative to the '540 patent's method, cffDNA can be used (i) without fractionation, or (ii) without amplification, or (iii) without distinguishing paternally-inherited DNA. *See* 9-11 *supra*. No other court resolving a Section 101 dispute — whether finding invalidity or patent-eligibility — has ever been presented with such concrete, real-world evidence of multiple alternative ways to use the same natural phenomenon or natural law but without practicing the allegedly "monopolizing" method. Sequenom's evidence

demonstrates the patent's non-preemptive effect and the District Court's refusal even to consider this evidence, Opinion at 18-20, was reversible error.

As this Court has stated:

> [T]he analysis under §101, while ultimately a legal determination, is rife with underlying factual issues. . . . *Likewise, any inquiry into the scope of preemption — how much of the field is 'tied up' by the claim — by definition will involve historic facts: identifying the 'field,' the available alternatives, and preemptive impact of the claims in that field.*

Ultramercial, 722 F.3d at 1339 (emphasis added).

The District Court disregarded Sequenom's evidence of alternative methods. *See* Opinion at 18-20. The District Court ruled that evidence of an alternative method would be relevant *only* if Sequenom demonstrated that the alternative method (i) already had been disclosed when the '540 patent was filed *and* (ii) is commercially viable. *Id*. at 19. The District Court cited no legal authority for its ruling, which contradicts Section 101 jurisprudence and public policy. This ruling was clear error.

In holding evidence proving *non*-preemptive uses to be irrelevant, the District Court overrode this Court's mandate that, to invalidate a patent under Section 101, there must be a "practical likelihood of a claim preempting a fundamental concept." *CLS*, 717 F.3d at 1277. Sequenom's evidence of peer-reviewed alternative uses of cffDNA rebuts any suggestion of a "practical likelihood" that the '540 patent's method monopolizes the use of fetal DNA and in

particular cffDNA. *See Ultramercial*, 722 F.3d at 1353 (claims are patent-eligible because "[t]here are myriad ways to accomplish that abstract concept that do not infringe these claims.").

Had the District Court considered Sequenom's evidence of alternative methods — as it should have done — it could not have held that there was clear and convincing proof that the '540 patent is manifestly ineligible as "preemptive."[3]

4. There Is No Rule Or Logic That Only "Previously Disclosed" Alternative Methods Are Relevant For Preemption Analysis.

The District Court noted that, as evidence of alternative methods, Sequenom presented three peer-reviewed articles published after the '540 patent was filed. Opinion at 19. From this, the District Court erroneously concluded:

> Therefore, even assuming that the articles disclose alternative methods of detecting cffDNA, Sequenom has failed to show that any alternative methods existed *at the time of the invention or at the time*

[3] Having ruled that Sequenom's evidence of alternative methods was irrelevant under its new requirements, the District Court then reached its preemption conclusion by relying on two tiny snippets extracted from statements non-legal Sequenom staff made to investors about its ability to block its competitors' products. Opinion at 19. The District Court deemed these two comments prove that "Sequenom has itself acknowledged the preemptive effect of its patent." *Id*. That was an unreasonable inference. The District Court did not allow for the comments' context, nor their intended audience, nor the speakers' non-legal training, nor that the competitors the speakers were referring to were Ariosa, Natera, and Verinata, who all copied the specific method of the '540 patent. At a minimum, these two snippets are less than clear and convincing evidence of preemption, especially when proper weight is given to Sequenom's countervailing evidence of alternative methods using cffDNA.

> *of issuance of the patent.* Thus, it appears that the effect of issuing the '540 patent was to wholly preempt all known methods of detecting cffDNA at that time.

Id. (emphasis added). The District Court's "previously disclosed" requirement defies precedent and offends public policy.

No precedent requires that non-preemptive methods must exist at the time of invention or patent issuance. To the contrary, the law is explicit that the concern is with patents that "tie up" the use of a natural phenomenon and "inhibit *future* innovation premised on them." *Myriad*, 133 S. Ct. at 2116 (emphasis added). *See also Mayo*, 132 S. Ct. at 1302 ("basic underlying concern [is] that these patents tie up too much *future use* of laws of nature"), 1302 (claims "covering all processes that make use of the correlations after measuring metabolites, including *later discovered* processes that measure metabolite levels in *new ways*"); *Morse*, 56 U.S. at 113 (concern is that a "*future* inventor, in the onward march of science, *may* discover" alternative means of using the natural law); *Benson*, 409 U.S. at 68 ("claim is so abstract and sweeping as to cover both known and *unknown* uses" of abstract idea) (emphases added throughout).

The '540 patent's claims do not tie up all uses of cffDNA nor foreclose future innovation, as demonstrated by the specific limitations of the patented method and Sequenom's evidence of three alternative methods. That these alternatives were first publicly disclosed after the '540 patent's filing date does not

diminish their relevance. The District Court's reliance on this fact contravenes the numerous explicit Supreme Court directives set forth above.

The District Court's "previously disclosed" requirement offends the public policy of encouraging inventors to apply for claims expeditiously. *See Transco Prods. Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 558 (Fed. Cir. 1994) (rejecting "rule [which] would subvert the patent system's goal of promoting the useful arts through encouraging early disclosure"). Such a requirement also offends the public policy of encouraging others to develop alternative methods following disclosure of the patented method. *See WMS Gaming Inc. v. Int'l Game Tech.*, 184 F.3d 1339, 1355 (Fed. Cir. 1999).

As the Supreme Court observed in *Myriad*, it is expected that the original discoverers of a natural phenomenon or law of nature will invent the first (and perhaps best) method of applying their discovery. *See* 133 S. Ct. at 2120. However, the District Court's rule would require inventors, such as Lo and Wainscoat — who discovered cffDNA in 1996 and claimed a method applying it a few months later — to wait, perhaps indefinitely, to patent their method until other inventors have disclosed alternative methods using the discovery.

Further, the District Court's "previously disclosed" requirement turns the presumption of validity on its head. Under the District Court's rule, the first-to-invent is branded as a "preempter" whose method is doomed to be patent-

ineligible. Because Lo and Wainscoat came up with the first-filed method applying their ground-breaking discovery of cffDNA before any alternatives had been published, under the District Court's reasoning, their patent necessarily preempts all other methods that could apply their discovery. Someone has to file first, and the first inventors should not be required to hold back disclosure of their method until others disclose alternative methods. The net effect of the waiting game the District Court's rule creates would be to stymie the disclosure and exploitation of inventions — the reverse of the incentives the patent laws are intended to foster. *See CLS*, 717 F.3d at 1281-82 ("What is needed is a flexible, pragmatic approach that can adapt and account for unanticipated technological advances while remaining true to the core principles underlying the fundamental exceptions to § 101.").

Preemption analysis is directed to whether a patent's claims are so broad and abstract that they preclude all other methods until the patent expires. New methods using a natural phenomenon are not all invented or revealed at the same time. Years after a discovery is first made, advances in technology or a new insight can spark the invention of an alternative method using the phenomenon in another or better way. Moreover, limitations, like those in the claims of the '540 patent, increase the likelihood that others have developed, or will develop, alternative methods.

When a patent is challenged as preemptive under Section 101, evidence of alternative methods — including methods disclosed after the patent was filed — *is* relevant to determining whether the patent is truly preemptive when challenged. Evidence of new, later-in-time alternative methods provides proof that the patent does not preempt all uses of the natural phenomenon.

5. The District Court's "Commercially Viable" Requirement Lacks Legal Basis And Contradicts Public Policy.

The District Court also mandated that, to be relevant on the issue of preemption, alternative methods must be shown to be "commercially viable." Opinion at 19. The "commercially viable" requirement would impose a higher standard on alternative, non-preemptive methods than now exists for *patented* methods. Neither law nor logic can justify this mismatch.

To be eligible for patenting generally, a claimed invention need only be useful and "provid[e] some identifiable benefit." *Juicy Whip, Inc. v. Orange Bang, Inc.*, 185 F.3d 1364, 1366 (Fed. Cir. 1999). There is no requirement that an invention be "commercially viable" to be patentable. *See CFMT, Inc. v. YieldUp Int'l Corp.*, 349 F.3d 1333, 1338 (Fed. Cir. 2003) ("Title 35 does not require that a patent disclosure enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment absent a claim limitation to that effect."); *Barmag Barmer Machinenfabrik AG v. Murata Machinery, Ltd.*, 731 F.2d 831, 839 (Fed. Circ. 1984) ("[C]ommercial marketability is not a requirement

of reduction to practice."). There is no logic in enforcing a standard for alternative methods offered to prove non-preemption that is more rigorous than the general law for patenting all other methods in all other contexts.

It is also irrational to base patent eligibility on whether the alternative method can maintain commercial traction. Under the District Court's reasoning, the Supreme Court should have invalidated Bell's telephone patents and Morse's telegraph claims because no commercially viable alternatives existed when their patents were filed or issued. No preemption-illuminating link is revealed by considering only those alternative methods which have fortuitously attracted the financial backing and managerial acumen necessary to sustain a commercially viable product.

Further, the District Court's standard has no factual mooring. The District Court gave no hint of what "commercial viability" means — does a failing, under-financed, or poorly-managed start-up count?; how profitable must a commercially viable competitor be?; how much of an otherwise successful diversified competitor's profits must come from the alternative method? The District Court's rule is as unworkable as it is misconceived.

The District Court's "commercially viable" standard would likely invalidate a first-filed patent teaching the *best* method of using a newly-discovered phenomenon. The market will winnow out inferior alternatives, eventually leaving

the market niche to the best application of the natural phenomenon. Yet, in these circumstances, rather than reward the inventor of the best method with a patent, the District Court's standard bars, or strips away, that patent as contrary to Section 101 because others are not commercially competitive.

This Court should reject the District Court's unprecedented and misconceived standard for determining what evidence is relevant for preemption analysis. The Court should reverse the District Court's rejection of Sequenom's compelling evidence of alternative methods. This Court's *de novo* review should give full weight to this evidence.

The undisputed evidence of at least these three alternative methods using cffDNA shows that the '540 patent does *not* preempt *all* uses of cffDNA in maternal blood. Each of the limitations in claims 1, 24, and 25 (and of the dependent claims) recites a combination of three essential steps using cffDNA, leaving the door open to other alternative methods applying the phenomenon. Those limitations are "meaningful." *Mayo*, 132 S. Ct. at 1302; *CLS*, 717 F.3d at 1281. This Court should conclude that there is no clear and convincing evidence that the '540 patent's method manifestly preempts all uses of cffDNA in maternal plasma or serum. Indeed, the relevant evidence is to the contrary. The Court should reverse for this reason alone.

C. The District Court Misconstrued The Meaning Of "Inventive Concept."

The District Court also committed error in holding that the '540 patent claimed patent-ineligible subject matter under Section 101 because the claims lacked an "inventive concept." Opinion at 14-15. The District Court reached this conclusion by misconstruing what the Supreme Court meant by an "inventive concept" inquiry in Section 101 eligibility analysis. Opinion at 15-17.

The District Court found no inventive concept because it decided that each element of the '540 patent's method, when separately considered, consisted only of "well-understood, routine and conventional activity by those in the field at the time of the invention." Opinion at 14. The Supreme Court has never equated "inventive concept" with the novelty or inventiveness of individual elements of a claimed method, but has instead explained the term as addressing whether the claims are sufficiently and meaningfully limited that the invention is not a claim on the natural phenomenon itself. *See Mayo*, 132 S. Ct. at 1294-97. The limitations recited in the '540 patent's method and the existence of several alternative methods using cffDNA demonstrate an inventive concept that crosses the Section 101 threshold.

Contrary to the District Court, "inventive concept" is a misnomer: under any reading of the Supreme Court's precedents, it does not require that the method, or any part of the method, be novel or inventive. *See Diehr*, 450 U.S. at 190 ("The

question therefore of whether a particular invention is novel is wholly apart from whether the invention falls into a category of statutory subject matter."). "We do not read the [Supreme] Court's occasional use of [inventive concept] in the § 101 context as imposing a requirement that such limitations must necessarily exhibit 'inventiveness'. . . ." *CLS*, 717 F.3d at 1282.

According to the *CLS* plurality opinion, "[a]n inventive concept in the § 101 context refers to a genuine human contribution to the claimed subject matter. . . . [A]n 'inventive concept' under § 101 — in contrast to whatever fundamental concept is also represented in the claim — must be a product of human ingenuity." *Id*. at 1283. The four-judge opinion by Judge Rader in *CLS* disagreed, finding no "ingenuity" requirement in Section 101. *Id*. at 1303 n.5. The '540 patent embodies an inventive concept whichever of these two views prevails because its method reflects a genuine human contribution that goes beyond the discovery of cffDNA by applying the discovery in a limited, useful, non-preemptive, and ingenious method of prenatal diagnosis.

In *Flook*, the Supreme Court "asked whether, to confer patent eligibility, the claim contained sufficient substance beyond the mathematical formula itself — that is, 'some other inventive concept in its application.'" *CLS*, 717 F.3d at 1278. (quoting *Flook*, 437 U.S. at 594). In *Mayo*, the Supreme Court cited to *Flook* and again referred to the need for an "inventive concept." *See* 132 S. Ct. at 1294.

In *Mayo*, the Supreme Court explained that "inventive concept" describes "other elements or a combination of elements" rendering the patent "significantly more than a patent upon the" prohibited subject matter alone. *Id*. at 1294. On this point, *Mayo* again relied on *Diehr*. The Supreme Court in *Diehr* had "found the overall process patent eligible because of the way the additional steps of the process integrated the equation into the process as a whole. . . . These other steps apparently added something to the formula that in terms of patent law's objectives had significance — they transformed the process into an inventive application of the formula." *Mayo*, 132 S. Ct. at 1298-99. In *Mayo*, as in *Flook*, the Supreme Court never suggested that the "other elements" must be novel or non-conventional or inventive. Rather, the method must not so closely embody the law of nature that the "inventive concept" recited in the patent was, in effect, the law of nature itself. *Id*. at 1294, 1297. If, as with the '540 patent, the method's limitations also reflect human ingenuity, *CLS*, 717 F.3d at 1283, Section 101 is amply satisfied.

Mayo "identified a two-step process." *Accenture*, 728 F.3d at 1341. Neither step focuses on whether the method's elemental techniques are novel or non-conventional. Instead, having first identified a patent-ineligible natural phenomenon, "the court must determine whether the claim poses 'any risk of preempting an abstract idea.'" *Id*. (quoting *CLS*, 717 F.3d at 1282 citing *Mayo*, 132 S. Ct. at 1302-03). The claim's limitations are "evaluated to determine

whether . . . [they] tie down the claim so that, in practical terms, it does not cover the full abstract idea itself." *Accenture*, 728 F.3d at 1341. *This* is the "inventive concept" determination, and, contrary to the District Court, it does not involve consideration of the novelty or conventionality of the method's elemental steps and techniques. In *Mayo*, the patent lacked any limitation over the relationship between the dose level and metabolite concentration. 132 S. Ct. at 1298. No doctor could administer thiopurine without using the relationship the patent claimed. *Id*. In *Mayo*, the patent consisted of the algorithm and nothing more, and thus lacked an inventive concept because it claimed a law of nature without meaningful limitations. *Id*.

Further, the District Court mistook *Mayo* and its reference to an "inventive concept" as requiring a bifurcating analysis: put the patent-ineligible matter to one side, and then scrutinize what remains for whether it "involves more than 'well-understood, routine, conventional activity' previously engaged in by those in the field." Opinion at 13-15. Once again, the District Court's analytical approach was off-base.

In *Diehr*, the examiner had rejected the patent because, in language reminiscent of the District Court here, the method's steps were "conventional and necessary to the process." 450 U.S. at 180-81. The Supreme Court disagreed. *Id*. at 193 n.15. "Those steps [in Diehr's patent] included steps that sound utterly old

and routine . . . . Indeed, even the Arrhenius equation was well-known in the art, but in combination was eligible." *CLS*, 717 F.3d at 1310. *Mayo* endorsed *Diehr*: "a new combination of steps in a process may be patentable even though all the constituents of the combination were well-known and in common use before the combination was made." *Mayo*, 132 S. Ct. at 1298 (quoting *Diehr*, 450 U.S. at 188). So too the '540 patent may rely on "steps that sound utterly old and routine" but in combination are patent-eligible.

The "conventional activity" in *Mayo* was the very method that Prometheus was trying to claim — administering the drug, measuring metabolite levels, and adjusting dosing based on the metabolite levels. 132 S. Ct. at 1297-98. Doctors were already doing just that before the patent, and could only continue this treatment by infringing the patent. *Id*. In contrast, before the '540 patent, *no one* was using the plasma or serum of pregnant mothers to amplify and detect paternally-inherited cffDNA. Indeed, what was "previously engaged in by those in the field" before the '540 patent was to throw away the maternal plasma and serum. *See Aria*, 726 F.3d at 1299. Unlike in *Mayo*, the '540 patent was a new combination and method.[4]

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[4] As a Congressional Committee recently stated:

> But most fundamentally, were the Committee to take seriously the suggestion that an invention is unpatentable if it adds "nothing of

(continued...)

The District Court declared that "[i]t is only an innovative or inventive use of a natural phenomenon that is afforded patent protection." Opinion at 15. As the Supreme Court and this Court have made clear, that is not so. *See Ultramercial*, 722 F.3d at 1348 ("The Supreme Court's reference to 'inventiveness' in *Mayo* can be read as shorthand for its inquiry into whether implementing the abstract idea in the context of the claimed invention inherently requires the recited steps.").

As shown above, using cffDNA does not inherently require, unlike the specific method in the '540 patent, the limited (and ingeniously combined) human interventions of fractionation, amplification, and detection of paternally-inherited DNA from cffDNA in maternal blood. The availability of several alternative peer-reviewed methods using cffDNA proves that each of these steps is a meaningful limitation of the '540 patent. Under *Mayo*, these limitations establish an inventive concept for Section 101 purposes. This Court should reverse for this reason also.

> significance" to the natural laws that control its operation, it must also conclude that the Patent Office should be deauthorized, for nothing would remain patentable other than whatever business methods survive [Supreme Court review].

HOUSE REPORT OF THE COMMITTEE ON THE JUDICIARY ON H.R. 3309 (THE INNOVATION ACT), H.R. REP. NO. 113-279, at 39 n.87 (2013).

D. The District Court Erred By Dissecting The Combined Method Of The '540 Patent Into Its Individual Elemental Techniques.

The District Court compounded its erroneous interpretation of the "inventive concept" requirement by conflating the distinct requirements of patent-eligibility and novelty into a myopic analysis of whether each individual step in the '540 patent's three-step method was "well-understood," "conventional," and "routine." Opinion at 13-15. The District Court's dissection of the elemental techniques that make up the method, rather than considering the combination of techniques as a whole as the Supreme Court has mandated, was a further reversible error.

It is irrelevant whether fractionation, amplification, and detection of nucleic acid, individually and in the abstract, were well-understood or routine laboratory techniques when the '540 patent was filed. What *is* probative of patent-eligibility, and what the District Court erroneously denied, was the patent's *combination* of these steps for the first time in a groundbreaking method for the purpose of detecting paternally-inherited cffDNA in maternal plasma or serum and diagnosing fetal characteristics. This new combination for this new purpose was neither conventional nor routine, and it satisfies the requirements of Section 101. *See CLS*, 717 F.3d at 1303 ("[A] new combination of old steps is patentable.") (Rader, C.J., concurring and dissenting).

Indeed, the Patent Act defines "process" as a "method, and includes a new use of a known process." 35 U.S.C. § 100(b). The '540 patent recites method

claims applying individual processes in combination to a new use — detecting and analyzing paternally-inherited cffDNA. *See Diehr*, 450 U.S. at 193 n.15 ("Invention was recognized because [the inventors] combined ordinary elements in an extraordinary way — a novel union of old means was designed to achieve new ends.").

The District Court reviewed each step of the '540 patent's method in isolation to determine whether, individually, it was a broadly accepted laboratory technique when the '540 patent was filed. *See* Opinion at 13-15. Because the District Court found that the laboratory techniques, individually, were not newly conceived as part of the invention, the District Court condemned the entire patent. *Id*. at 15. The District Court's methodology contravened a key tenet of Section 101 law — it improperly dissected the method into its constituent parts, overriding the Supreme Court's direction that the method be considered only as a whole.

In *Diehr*, the Supreme Court held that conventional steps, when combined for a new purpose, can constitute a patent-eligible method. 450 U.S. at 180-81, 193 n.15. "The fact that one or more steps in respondents' process may not, in isolation, be novel or independently eligible for patent protection is irrelevant to the question of whether the claims *as a whole* recite subject matter *eligible* for patent protection under § 101." *Id*. at 193 n.15 (original emphasis).

The District Court should have looked at whether the techniques' *combination* for fractionating, amplifying, and detecting paternally-inherited cffDNA in maternal blood to diagnose fetal characteristics involved an inventive concept (as discussed above) when considered *as a whole*. As the Supreme Court held:

> It is inappropriate to dissect the claims into old and new elements and then to ignore the presence of the old elements in the analysis. This is particularly true in a process claim because a new combination of steps in a process may be patentable even though all the constituents of the combination were well known and in common use before the combination was made.

Diehr, 450 U.S. at 188. A properly focused, non-dissecting examination of the '540 patent's entire method shows it is patent-eligible.

The District Court maintained that it considered whether the combination of techniques was conventional when the patent was filed, and cited evidence it said supported this conclusion. Opinion at 18. This evidence is neither clear nor convincing. The cited evidence refers specifically to diagnosing *cancer* from DNA derived from plasma or serum, rather than using paternally-inherited cffDNA in maternal blood to detect fetal characteristics. *Id*. at 14; A0197, ¶ 58; A0199 ¶ 69. Among the many marked differences between the '540 patent and the prior art the District Court cited are those relating to who is being tested (cancer patients of all genders and ages vs. pregnant women) and to what is being detected (tumors vs. fetal characteristics). Thus, in this respect also, the District Court erred.

The ’540 patent claims a specific and meaningfully limited use of paternally-inherited cffDNA in maternal plasma or serum by applying an original combination of steps. “In sum, as a practical application of the general concept . . ., the claimed invention is not ‘so manifestly abstract as to override the statutory language of section 101.’” *Ultramercial*, 722 F.3d at 1354.

E. *Myriad* Supports The Eligibility Of The Invention Claimed In The ’540 Patent.

When, in August 2013, this Court decided the *Aria* case, it vacated the District Court’s finding of “a substantial question” as to whether the ’540 patent satisfies Section 101. *See Aria*, 726 F.3d at 1304. The Court directed the District Court to re-consider its Section 101 ruling “in light of [*Myriad*].” *Id*. In *Myriad*, the Supreme Court provided an explicit analytical framework for determining whether claims involving a natural phenomenon are patent-eligible. In its subsequent summary judgment decision three months later, the District Court misapprehended and misapplied *Myriad*. This further error also requires reversal.

1. Claims To A Laboratory-Transformed Variant Of A Natural Phenomenon, Such As Amplified cffDNA, Are Patent-Eligible.

In *Myriad*, the Supreme Court considered whether the natural phenomenon exception to Section 101 applied to composition claims to isolated sequences of the BRCA-1 and BRCA-2 genes. *See* 133 S. Ct. at 2119. These composition claims were unlimited as to use. The claims “would, if valid, give [Myriad] the

exclusive right to isolate an individual's BRCA1 and BRCA2 genes . . . ." *Id*. at 2113. The Court found that "[s]eparating that gene from its surrounding genetic material is not an act of invention." *Id*. at 2117.

On the other hand, the Supreme Court held that Myriad's claim to a laboratory-based composition isolating complementary DNA "does not present the same obstacles to patentability as naturally occurring, isolated DNA segments." *Id*. at 2119 ("cDNA is not a 'product of nature'"). Creating cDNA was "an act of invention." *Id*. "[T]he lab technician unquestionably creates something new when cDNA is made." *Id*.

Thus, in *Myriad*, the Supreme Court drew the patent-ineligibility line tightly around the genes' DNA sequences themselves. Composition claims to non-naturally-occurring material created from those sequences by a conventional method, such as the routine laboratory work involved in making cDNA, were held to be patent-eligible. *Id*. The laboratory technique of amplifying cffDNA is analogous to making cDNA.

In nature, DNA is transcribed into RNA, and then into mRNA. *Id*. at 2111-12. cDNA is laboratory-made from the naturally-occurring mRNA: "cDNA is synthesized from mRNA using complementary base pairing in a manner analogous to RNA transcription." *Ass'n for Molecular Pathology v. United States Patent and Trademark Office*, 689 F.3d 1303, 1313 (Fed. Cir. 2012), *aff'd in part, rev'd in*

part, *Myriad*, 133 S. Ct. 2107. Similar to PCR amplification, the cDNA laboratory process uses an enzyme to make a strand that is a complementary copy of the mRNA, and another enzyme to make a second strand complementary to the first strand. This "results in a double-stranded DNA molecule with a sequence corresponding to the sequence of an mRNA produced by the body." *Id*.

Myriad holds that even a small step away from the natural phenomenon as the result of human intervention is sufficient for patent-eligibility. The patent-eligible cDNA molecules Myriad produced do not exist in nature, even though the sequence information in the cDNA is the same as it exists in nature. *Myriad*, 133 S. Ct. at 2111, 2116, 2119. "The nucleotide sequence of cDNA is dictated by nature, not by the lab technician." *Id*. at 2119. The only substantive difference is a single base nucleotide — a nucleotide base thymine ("T") in cDNA in place of a uracil ("U") in the original mRNA. *Id*. at 2111. Thus, *Myriad* holds that producing material that differs from that found in nature because a technician has applied a conventional laboratory technique to the naturally-occurring matter satisfies the patent-eligibility threshold of Section 101.

Unlike the unbounded composition claim in *Myriad*, a method claim includes limitations. By analogy, the limited method claims of the '540 patent create synthetic cffDNA by producing DNA strands that differ from the natural phenomenon that is, as with cDNA, a "*new application* [] of knowledge,"

"something new," and patent-eligible. *Id.* at 2119, 2120. "Transformation and reduction of an article 'to a different state or thing' is the clue to patentability of a process claim that does not include particular machines." *Benson*, 409 U.S. at 70.

Amplified cffDNA is physically and chemically distinct from naturally-occurring cffDNA. *See* pages 7-9 *supra*. In the first round of PCR, when the primers attach to the natural DNA at their complementary targets and amplify the DNA sequences between those targets, a physically longer (or shorter, if universal PCR is not used) segment is produced. Similarly, laboratory-produced amplified cffDNA has no methylated CpG sites, unlike almost all naturally-occurring cffDNA. *See id.*

When *Myriad* created cDNA, it applied a well-understood laboratory technique to the naturally-occurring BRCA gene to create patent-eligible cDNA from those sequences. *See Myriad*, 133 S. Ct. at 2112, 2119-20. Similarly, the '540 patent's method applies a combination of known laboratory techniques to naturally-occurring cffDNA to create patent-eligible amplified cffDNA sequences which then must be detected through additional laboratory manipulation.

2. Methods Applying Known Laboratory Techniques To A Newly-Discovered Natural Phenomenon, As In Myriad's Claim 21, Are Patent-Eligible.

The *Myriad* opinion provides another significant patent-eligibility guidepost. The Supreme Court indicated how it might have decided the patent-eligibility of Myriad's *method* claims had they also been challenged under Section 101:

> Similarly, this case does not involve patents on new *applications* of knowledge about the BRCA1 and BRCA2 genes. Judge Bryson aptly noted that, "[a]s the first party with knowledge of the [genes'] sequences, Myriad was in an excellent position to claim applications of that knowledge. Many of its unchallenged claims are limited to such applications."

Id. at 2120 (original emphasis) (quoting *Ass'n for Molecular Pathology*, 689 F.3d at 1349 (Bryson, J., concurring and dissenting)).

The "apt" portion of Judge Bryson's opinion referenced several "unchallenged claims." *See* 689 F.3d at 1349. Judge Bryson specifically identified claim 21 of Myriad's '441 patent as one such "unchallenged claim." *Id.* Myriad's claim 21 in its '441 patent recites a method for detecting a BRCA1 gene mutation:

> The method of claim 20 wherein a germline alteration is detected by hybridizing a BRCA1 gene probe which specifically hybridizes to an allele of one of said alterations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said allele in the sample.

See RJN at 3-5, Malecek Dec., ¶ 2 & Exh. A. Contrary to the District Court, Opinion at 15 n. 8, this Court has confirmed that "hybridizing" gene "probes" was a well-established technique long before Myriad's patent was filed. *See Enzo*

Biochem, Inc. v. Applera Corp., 599 F.3d 1325, 1328-29 (Fed. Cir. 2010) (discussing "hybridization" and "probe" techniques in a 1988 patent); *see also* RJN at 4-9 & Malecek Dec., ¶¶ 3-4 & Exhs. B, C (scientific treatises describing conventionality in 1989 of techniques recited in Claim 21).

Despite the Supreme Court's positive reference to Judge Bryant's "apt" statement, the District Court rejected any reference to Myriad's claim 21 because "the Supreme Court did not refer to claim 21." Opinion at 17 n.8. However, the Supreme Court went out of its way to discuss the likely Section 101 eligibility of method patents applying a natural phenomenon, quoting approvingly Judge Bryson's "apt" statement from his dissent in this Court. Because claim 21 was among the claims Judge Bryson cited in the passage the Supreme Court adopted, *see* 689 F.3d at 1349, it must have been among the potentially patent-eligible "applications" the Supreme Court had in mind. 133 S. Ct. at 2120. The District Court should not have dismissed the essential point the Supreme Court was making through Judge Bryson — that method claims like Myriad's unchallenged claim 21 are likely be patent-eligible under Section 101.

Comparing Myriad's claim 21 with claim 1 of the '540 patent is instructive. Both claims recite a combination of conventional techniques applied to a natural phenomenon. Myriad discovered that a variant of the BRCA gene is associated with breast cancer. Its claim 21 described a method for detecting that mutation

involving no more than hybridizing a probe to detect whether the variant is present. This method is text-book conventional and routine laboratory work, *see* RJN at 4-9 & Malecek Dec., ¶¶ 3-4 & Exhs. B, C, yet the Supreme Court has implied claim 21 claims patent-eligible subject matter. If, as the Supreme Court at least implied, Myriad's claim 21 is patent-eligible, then the combination of techniques described in the method recited in the '540 patent should likewise pass Section 101's threshold.

For this reason also, this Court should hold that the '540 patent is eligible under Section 101, and should reverse the District Court.

CONCLUSION

For the reasons set forth above, this Court should reverse. The Court should hold that the '540 patent describes a patent-eligible method satisfying Section 101.

Dated: January 21, 2014

Respectfully submitted,
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CERTIFICATE OF SERVICE

I, Michael J. Malecek, counsel for Appellant and a member of the Bar of this Court, certify that, on January 17, 2014, counsel of record for Appellees Ariosa Diagnostics, Inc., Natera, Inc., and Verinata Health, Inc. were each served a copy of the Opening Brief of Appellant Sequenom, Inc. by electronic means through CM/ECF. I further certify that all parties required to be served have been served.

Dated: January 21, 2014

/s/ Michael J. Malecek
Michael J. Malecek
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CERTIFICATE OF COMPLIANCE

1. This brief complies with the type-volume limitation of Federal Rule of Appellate Procedure 32(a)(7)(B). The brief contains 13,226 words, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(a)(7)(B)(iii) and Federal Circuit Rule 32(b).

2. This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6). The brief has been prepared in a proportionally spaced typeface using Microsoft Word 2007 in 14-point Times New Roman font.

Dated: January 21, 2014

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Sequenom, Inc.

ADDENDUM

ADDENDUM TABLE OF CONTENTS

1

IN THE UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

ARIOSA DIAGNOSTICS, INC.,

Plaintiff/Counterdefendant,

v.

SEQUENOM, INC.,

Defendant/Counterclaimant.
____/

No. C 11-06391 SI

ORDER GRANTING PLAINTIFF'S MOTION FOR SUMMARY JUDGMENT AND DENYING DEFENDANT'S MOTION FOR SUMMARY JUDGMENT

Cross-motions for summary judgment by plaintiff/counterdefendant Ariosa Diagnostics, Inc. and defendant/counterclaimant Sequenom, Inc. came on for oral argument on October 11, 2013. Having considered the parties' motion papers, pleadings and arguments, and for good cause shown, the Court GRANTS Ariosa's motion for summary judgment and DENIES Sequenom's motion for summary judgment.

BACKGROUND

In this declaratory judgment action, plaintiff Ariosa, formerly known as Aria Diagnostics, Inc., seeks a declaration that its non-invasive prenatal test, the Harmony test, using cell-free fetal DNA circulating in the blood of a pregnant woman does not directly infringe or contribute to the infringement of U.S. Patent No. 6,258,540 ("the '540 patent"), licensed by defendant Sequenom.

1. The '540 Patent

Sequenom is the exclusive licensee of the '540 patent, which Sequenom licensed from Isis Innovation Limited ("Isis"). *See* Docket No. 37, Tatman Decl. ¶¶ 3-4. The '540 patent is entitled "Non-Invasive Prenatal Diagnosis," and was issued to inventors Yuk-Ming Dennis Lo and James Stephen Wainscoat on July 10, 2001 and assigned to Isis. U.S. Patent No. 6,258,540 The '540 patent relates to prenatal detection methods performed on a maternal serum or plasma sample from a pregnant female, which methods comprise detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample. *Id.* at 2:1-4. "This invention enables non-invasive prenatal diagnosis, including for example sex determination, blood typing and other genotyping, and detection of pre-eclampsia in the mother." *Id.* (Abstract).

According to the patent, conventional pre-natal diagnostic DNA tests such as amniocentesis and chorionic villus sampling involved invasive procedures with risks to the mother and the pregnancy. '540 Patent at 1:12-17; *see also* Docket No. 35, Evans Decl. ¶¶ 34-37. Therefore, non-invasive techniques began to be developed that used maternal blood or serum. '540 Patent at 1:18-20. Prior non-invasive DNA research had focused on detecting fetal cells in a mother's bloodstream, because the presence of cell-free fetal DNA was not known. *Id.* at 1:28-36; *see also* Docket No. 35, Evans Decl. ¶ 21. However, these techniques were time-consuming or required expensive equipment. '540 Patent at 1:36-37; *see also* Docket No. 35, Evans Decl. ¶¶ 39-41 ("Ultimately, neither approach, using fetal cells or the other noninvasive screening measurements described above, has proved sufficiently successful or reliable to replace invasive testing.").

The '540 patent is based on the discovery in 1996-1997 by Drs. Lo and Wainscoat that cell-free fetal DNA (sometimes referred to as "cffDNA") is detectable in maternal serum or plasma samples.[1] '540 Patent at 1:50-51; *see also* Docket No. 35, Evans Decl. ¶ 45. This discovery was important

[1] "Nucleic acid" is the overall name for the class of molecules that includes DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). The significance of the discovery is that the process of isolating fetal cells was not necessary because fetal DNA was present outside of cells, as "extracellular" or "cell-free DNA" suspended in the maternal bloodstream. Docket No. 35, Evans Decl. ¶¶ 53, 57. Blood is made up of cells and plasma (the fluid containing proteins and other molecules in which cells are suspended). *Id.* ¶ 44. Serum is plasma without the clotting proteins (platelets), *i.e.*, blood minus the cells and the clotting factors. *Id.*

because according to the patent, "[t]he detection rate is much higher using serum or plasma than using nucleated blood cell DNA extracted from a comparable volume of whole blood, suggesting there is enrichment of foetal DNA in maternal plasma and serum." '540 Patent at 1:55-58.

The three independent claims of the '540 patent are as follows:

> **1.** A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.
>
> **24.** A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:
> removing all or substantially all nucleated and anucleated cell populations from the blood sample,
> amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally [sic] inherited fetal nucleic acid.
>
> **25.** A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises
> obtaining a non-cellular fraction of the blood sample
> amplifying a paternally inherited nucleic acid from the non-cellular fraction and
> performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal nucleic acid.

'540 Patent at 23:60-67; 26:20-36.

2. Procedural Background

Ariosa filed this declaratory relief action against Sequenom on December 19, 2011, seeking a declaration that its Harmony Test does not infringe any claims of the '540 patent.[2] Docket No. 1, Compl. On March 8, 2012, Sequenom filed an answer against Ariosa and a counterclaim for infringement of the '540 patent. Docket No. 33. On March 8, 2012, Sequenom also filed a motion for a preliminary injunction, seeking to enjoin Ariosa from making, using, selling, offering for sale, or importing into the United States the Harmony Prenatal Test. Docket No. 34.

On July 5, 2012, the Court denied Sequenom's motion for a preliminary injunction. Docket No. 121. In the order, the Court found that Ariosa had raised a substantial question with regard to the

[2] Two other cases have been filed in the Northern District of California which also seek declaratory judgments that specific products do not infringe the '540 patent and that the '540 patent is invalid. *See Natera, Inc. v. Sequenom, Inc.*, Case No. 12-cv-00132-SI (filed Jan. 6, 2012); *Verinata Health, Inc. v. Sequenom, Inc.*, Case No. 12-cv-865-SI (filed Feb. 22, 2012).

validity of the '540 patent based on Ariosa's argument that the '540 patent does not cover patent eligible subject matter. *Id.* at 16-19. Sequenom appealed the Court's denial of its motion for a preliminary injunction. Docket No. 123.

On August 9, 2013, the Federal Circuit vacated the Court's order denying the preliminary injunction and remanded the case for further proceedings. *Aria Diagnostics, Inc. v. Sequenom, Inc.*, 726 F.3d 1296, 2013 U.S. App. LEXIS 16506 (Fed. Cir. 2013). In vacating the order, the Federal Circuit rejected this Court's initial claim construction, but offered no opinion as to whether there is or is not a substantial question regarding the subject matter eligibility of the asserted claims of the '540 patent. *Id.* at *16-17. Rather, the Federal Circuit remanded with directions that this Court examine subject matter eligibility of the asserted claims in the first instance in light of the Supreme Court's recent decision in *Association for Molecular Pathology v. Myriad Genetics, Inc.*, 133 S. Ct. 2107 (2013) and the Federal Circuit's claim construction holdings. *Id.* at *16.

By the present cross-motions for summary judgment, the parties move for summary adjudication of whether claims 1, 2, 4, 5, 8, 19-22, 24, and 25 of '540 patent are drawn to patent-eligible subject matter.

LEGAL STANDARD

1. Summary Judgment

Summary judgment is proper "if the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law." Fed. R. Civ. P. 56(a). The moving party bears the initial burden of demonstrating the absence of a genuine issue of material fact. *Celotex Corp. v. Catrett*, 477 U.S. 317, 323 (1986). The moving party, however, has no burden to disprove matters on which the non-moving party will have the burden of proof at trial. The moving party need only demonstrate to the Court that there is an absence of evidence to support the non-moving party's case. *Id.* at 325.

Once the moving party has met its burden, the burden shifts to the nonmoving party to "set forth, by affidavit or as otherwise provided in Rule 56, 'specific facts showing that there is a genuine issue for trial.'" *T.W. Elec. Service, Inc. v. Pacific Elec. Contractors Ass'n*, 809 F.2d 626, 630 (9th Cir. 1987)

(citing *Celotex*, 477 U.S. at 324). To carry this burden, the non-moving party must "do more than simply show that there is some metaphysical doubt as to the material facts." *Matsushita Elec. Indus. Co., Ltd. v. Zenith Radio Corp.*, 475 U.S. 574, 586 (1986). "The mere existence of a scintilla of evidence . . . will be insufficient; there must be evidence on which the jury could reasonably find for the [non-moving party]." *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 252 (1986).

In deciding a summary judgment motion, the Court must view the evidence in the light most favorable to the non-moving party and draw all justifiable inferences in its favor. *Id.* at 255. "Credibility determinations, the weighing of the evidence, and the drawing of legitimate inferences from the facts are jury functions, not those of a judge . . . ruling on a motion for summary judgment." *Id.* However, conclusory, speculative testimony in affidavits and moving papers is insufficient to raise genuine issues of fact and defeat summary judgment. *Thornhill Publ'g Co., Inc. v. GTE Corp.*, 594 F.2d 730, 738 (9th Cir. 1979). The evidence the parties present must be admissible. Fed. R. Civ. P. 56(c)(2).

2. Subject Matter Eligibility Under § 101

Under § 101 of the Patent Act, "[w]hoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title." 35 U.S.C. § 101. "In choosing such expansive terms . . . modified by the comprehensive 'any,' Congress plainly contemplated that the patent laws would be given wide scope." *Diamond v. Chakrabarty*, 447 U.S. 303, 308 (1980).

However, the Supreme Court has long held that there is an important exception to § 101: "'[L]aws of nature, natural phenomena, and abstract ideas' are not patentable." *Mayo Collaborative Servs. v. Prometheus Labs., Inc.*, 132 S. Ct. 1289, 1293 (2012); *see also id.* ("[T]he [Supreme] Court has written that a new mineral discovered in the earth or a new plant found in the wild is not patentable subject matter. Likewise, Einstein could not patent his celebrated law that $E=mc^2$; nor could Newton have patented the law of gravity. Such discoveries are manifestations of . . . nature, free to all men and reserved exclusively to none." (internal quotation marks omitted)). The Federal Circuit has explained that these exceptions should be applied narrowly. *Ultramercial, Inc. v. Hulu, LLC*, 722 F.3d 1335, 1342

(Fed. Cir. 2013); *see also Prometheus*, 132 S. Ct. at 1293 ("The Court has recognized . . . that too broad an interpretation of this exclusionary principle could eviscerate patent law. For all inventions at some level embody, use, reflect, rest upon, or apply laws of nature, natural phenomena, or abstract ideas.").

Patent eligibility under § 101 is an issue of law that may involve underlying factual issues. *Accenture Global Servs. v. Guidewire Software, Inc.*, 2013 U.S. App. LEXIS 18446, at *10 (Fed. Cir. Sept. 5, 2013). Moreover, under 35 U.S.C. § 282, patents are presumed to be valid. Therefore, an alleged infringer must prove invalidity by clear and convincing evidence. *See Microsoft Corp. v. i4i L.P.*, 131 S. Ct. 2238, 2242 (2011); *see also Ultramercial*, 722 F.3d at 1339 (explaining that an accused infringer must prove ineligible subject matter under § 101 by clear and convincing evidence). In this connection, it is the factual evidence itself which must be clear and convincing. *See Buildex, Inc. v. Kason Indus., Inc.*, 849 F.2d 1461, 1463 (Fed. Cir. 1988) (clear and convincing evidence is evidence "which produces in the mind of the trier of fact an abiding conviction that the truth of [the] factual contentions are highly probable" (alteration in original) (citation and internal quotation marks omitted)).

3. Supreme Court Case Law on Subject Matter Eligibility

The Supreme Court has issued several recent decisions articulating standards for the subject matter eligibility, building on cases decided over the last half-century. Several of these cases are briefly reviewed below.

A. *Funk Brothers*

The patent in *Funk Brothers* claimed an inoculant for leguminous plants comprising a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium, where the strains are unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.[3] *Funk Bros. Seed Co. v. Kalo Inoculant Co.*, 333 U.S. 127, 129 n.3

[3] Leguminous plants take nitrogen from the air and fix it in the plant for conversion to organic nitrogenous compounds. *Funk Bros.*, 333 U.S. at 129. The ability of these plants to fix nitrogen from the air depends on the presence of bacteria of the genus Rhizobium in the plant. *Id.* Bacteria of the genus Rhizobium fall into at least six species. *Id.* "No one species will infect the roots of all species of leguminous plants. But each will infect well-defined groups of those plants." *Id.*

(1948). The Supreme Court noted that prior to the invention, the general practice was to manufacture and sell inoculants containing only one of the six species of the Rhizobium bacteria, meaning that the inoculant could only be used successfully in plants that belonged to that specific species' inoculation group. *Id.* at 129. The inventors of the patent discovered that there are strains of each species of bacteria which do not exert a mutually inhibitive effect on each other, and, therefore, could be isolated and used in mixed cultures. *Id.* at 130. "Thus [the invention] provided a mixed culture of Rhizobia capable of inoculating the seeds of plants belonging to several cross-inoculation groups." *Id.*

The Supreme Court held that the claims were not patentable because "patents cannot issue for the discovery of the phenomena of nature." *Id.* at 130. The Supreme Court explained that discovery of the fact that certain strains of each species of these bacteria can be mixed without harmful effect to the properties of either is no more than the discovery of some of the handiwork of nature and hence is not patentable. *Id.* at 131. "If there is to be invention from such a discovery, it must come from the application of the law of nature to a new and useful end." *Id.* at 130. The Court recognized that the aggregation of select strains of the species of bacteria into one product is an application of a newly-discovered natural principle, but explained that the application of that principle "is hardly more than an advance in the packaging of the inoculants." *Id.* at 131; *see also id.* at 132 ("[O]nce nature's secret of the non-inhibitive quality of certain strains of the species of Rhizobium was discovered, the state of the art made the production of a mixed inoculant a simple step.").

B. *Gottschalk v. Benson*

The patent application in *Benson* "claimed a method for converting binary-coded decimal (BCD) numerals into pure binary numerals." *Gottschalk v. Benson*, 409 U.S. 63, 64 (1972). The Supreme Court noted that "[t]he claims were not limited to any particular art or technology, to any particular apparatus or machinery, or to any particular end use," and "[t]hey purported to cover any use of the claimed method in a general-purpose digital computer of any type." *Id.*; *see also id.* at 68 ("Here the 'process' claim is so abstract and sweeping as to cover both known and unknown uses of the BCD to pure binary conversion").

The Supreme Court held that the claims were ineligible subject matter because the formula for

converting BCD numerals to pure binary numerals was an abstract idea. *See id.* at 71. The Court explained: "The mathematical formula involved here has no substantial practical application except in connection with a digital computer, which means that if the judgment below is affirmed, the patent would wholly pre-empt the mathematical formula and in practical effect would be a patent on the algorithm itself." *Id.* at 71-72.

C. *Parker v. Flook*

The patent application in *Flook* claimed a method of updating alarm limits,[4] consisting of three steps: "an initial step which merely measures the present value of the process variable (e.g., the temperature); an intermediate step which uses an algorithm to calculate an updated alarm-limit value; and a final step in which the actual alarm limit is adjusted to the updated value." *Parker v. Flook*, 437 U.S. 584, 585 (1978). The Court noted that "[t]he only difference between the conventional methods of changing alarm limits" and the claimed method "rests in the second step – the mathematical algorithm or formula." *Id.* at 585-86; *see also id.* at 588 (stating that because the patentee did not challenge the examiner's finding, the Court assumed that "the formula is the only novel feature of respondent's method").

The Supreme Court held that the application did not claim a patentable invention. *Id.* at 594. The Supreme Court explained that "[t]he only novel feature of the method is a mathematical formula," *id.* at 585, and the discovery of a phenomenon of nature or mathematical formula "cannot support a patent unless there is some other inventive concept in its application." *Id.* at 594. In addition, the Supreme Court rejected the patentee's argument that his invention was patentable because, unlike the patent in *Benson*, his invention did not wholly preempt the use of a mathematical formula. *See id.* at 589-95. The Court recognized that the invention did not wholly preempt the formula, but explained that

[4] "An 'alarm limit' is a number." *Parker v. Flook*, 437 U.S. 584, 585 (1978). During catalytic conversion processes (various processes used in the petrochemical and oil-refining industries), operating conditions such as temperature, pressure and flow rates are constantly monitored. *Id.* "When any of these 'process variables' exceeds a predetermined 'alarm limit,' an alarm may signal the presence of an abnormal condition indicating either inefficiency or perhaps danger. Fixed alarm limits may be appropriate for a steady operation, but during transient operating situations, such as start-up, it may be necessary to 'update' the alarm limits periodically." *Id.*

"'if a claim is directed essentially to a method of calculating, using a mathematical formula, even if the solution is for a specific purpose, the claimed method is nonstatutory.'" *Id.* at 595 (quoting *In re Richman*, 563 F.2d 1026, 1030 (CCPA 1977)); *see also id.* at 590 ("The notion that post-solution activity, no matter how conventional or obvious in itself, can transform an unpatentable principle into a patentable process exalts form over substance. A competent draftsman could attach some form of post-solution activity to almost any mathematical formula; the Pythagorean theorem would not have been patentable, or partially patentable, because a patent application contained a final step indicating that the formula, when solved, could be usefully applied to existing surveying techniques.").

D. *Diamond v. Diehr*

The patent application in *Diehr* claimed "a process for molding raw, uncured synthetic rubber into cured precision products." *Diamond v. Diehr*, 450 U.S. 175, 177 (1981). The process involved constantly determining the actual temperature inside the mold, then automatically feeding the temperatures into a computer which would repetitively calculate the necessary cure time using a mathematical formula known as the Arrhenius equation, and opening the press whenever the elapsed cure time equaled the calculated necessary cure time. *See id.* at 178-79 & n.5.

The Supreme Court found the invention to be patentable. The Court held that "a physical and chemical process for molding precision synthetic rubber products falls within the § 101 categories of possibly patentable subject matter." *Id.* at 184. The Court distinguished the invention at issue from the inventions found unpatentable in *Benson* and *Flook*. *See id.* at 185-88, 191-92 & n.14. The Court recognized that "the process admittedly employs a well-known mathematical equation, but [the patentees] do not seek to pre-empt the use of that equation. Rather, they seek only to foreclose from others the use of that equation in conjunction with all of the other steps in their claimed process." *Id.* at 187. "[W]hen a claim containing a mathematical formula implements or applies that formula in a structure or process which, when considered as a whole, is performing a function which the patent laws were designed to protect (e. g., transforming or reducing an article to a different state or thing), then the claim satisfies the requirements of § 101." *Id.* at 192. In addition, unlike in *Flook*, the patentees contended that there were novel aspects of the invention other than the use of the mathematical formula.

See id. at 178-79.

E. *Bilski v. Kappos*

The patent application in *Bilski* claimed a procedure for instructing buyers and sellers of commodities in the energy market how to protect against the risk of price fluctuations in those commodities. *Bilski v. Kappos*, 130 S. Ct. 3218, 3223 (2010). "Claim 1 describes a series of steps instructing how to hedge risk. Claim 4 puts the concept articulated in claim 1 into a simple mathematical formula. . . . The remaining claims explain how claims 1 and 4 can be applied to allow energy suppliers and consumers to minimize the risks resulting from fluctuations in market demand for energy." *Id.* at 3223-24.

The Supreme Court held that the claims were unpatentable under *Benson*, *Flook*, and *Diehr* because the claims "are attempts to patent abstract ideas." *Id.* at 3230. The Court explained that claims 1 and 4 in the patentees' application explain the basic concept of hedging, or protecting against risk, and the concept of hedging is an unpatentable abstract idea. *Id.* at 3231. "Allowing petitioners to patent risk hedging would preempt use of this approach in all fields, and would effectively grant a monopoly over an abstract idea." *Id.* The Court also rejected the remaining claims of the application because they were "broad examples of how hedging can be used in commodities and energy markets." *Id.* "*Flook* established that limiting an abstract idea to one field of use or adding token postsolution components d[o] not make the concept patentable." *Id.*

F. *Mayo v. Prometheus*

The patents in *Prometheus* claimed processes that help doctors using thiopurine drugs to treat patients with autoimmune diseases determine whether a given dosage level is too low or too high. *Prometheus*, 132 S. Ct. at 1294. Too high a dosage would risk harmful side effects, but too low a dosage might be ineffective. *Id.* at 1295. At the time of the invention, scientists already understood that the levels of certain metabolites in a patient's blood were correlated with the likelihood that a particular dosage of a thiopurine drug could cause harm or prove ineffective. *Id.* The patents' claims set forth processes embodying researchers' findings that identified the precise correlations between metabolite

levels and likely harm or ineffectiveness. *Id.*

The Supreme Court held that the claims were invalid under § 101. *Id.* at 1305. The Court explained that "Prometheus' patents set forth laws of nature – namely, relationships between concentrations of certain metabolites in the blood and the likelihood that a dosage of a thiopurine drug will prove ineffective or cause harm." *Id.* at 1296. "If a law of nature is not patentable, then neither is a process reciting a law of nature, unless that process has additional features that provide practical assurance that the process is more than a drafting effort designed to monopolize the law of nature itself. A patent, for example, could not simply recite a law of nature and then add the instruction 'apply the law.'" *Id.* at 1297. Therefore, the Court concluded that although the patents recited additional steps in addition to the law of nature, the additional steps were insufficient to transform the character of the claims. *See id.* at 1297-98 ("[T]he claims inform a relevant audience about certain laws of nature; any additional steps consist of well understood, routine, conventional activity already engaged in by the scientific community; and those steps, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.").

G. *Ass'n for Molecular Pathology v. Myriad*

The patentees in *Myriad* discovered the precise location and sequence of two human genes, the BRCA1 and BRCA2 genes, mutations of which can substantially increase the risks of breast and ovarian cancer, and obtained several patents based on that discovery. *Myriad*, 133 S. Ct. at 2110-11. The claims at issue gave Myriad "the exclusive right to isolate an individual's BRCA1 and BRCA2 genes . . . by breaking the covalent bonds that connect the DNA to the rest of the individual's genome. The patents [also gave] Myriad the exclusive right to synthetically create BRCA cDNA [("complementary DNA")]." *Id.* at 2113.

The Supreme Court held that "a naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated, but that cDNA is patent eligible because it is not naturally occurring." *Id.* at 2111. The Court noted that Myriad did not create or alter any of the genetic information encoded in the BRCA1 and BRCA2 genes and did not create or alter the genetic structure of DNA. *Id.* at 2116. "Instead, Myriad's principal contribution was uncovering the precise location and

genetic sequence of the BRCA1 and BRCA2 genes within chromosomes 17 and 13." *Id.* "To be sure, [Myriad] found an important and useful gene, but separating that gene from its surrounding genetic material is not an act of invention." *Id.* at 2117. In contrast, the Court found that cDNA is not a "product of nature" and, therefore, is patent eligible under § 101. *Id.* at 2119.

DISCUSSION

Ariosa argues that claims 1, 2, 4, 5, 8, 19-22, 24, and 25 of the '540 patent are not drawn to patent eligible subject matter because paternally inherited cffDNA is a natural phenomenon and the claims of the '540 patent merely add well-understood, routine, conventional activity in the field to that natural phenomenon. Docket No. 219 at 7-20. In response, Sequenom argues that the claimed methods are patentable because they are novel uses of a natural phenomenon, rather than a patent on the natural phenomenon itself. Docket No. 223 at 7-18. In addition, Sequenom argues that the claims are patentable because the claims do not preempt all uses of cffDNA. *Id.* at 18-22.

The parties agree that neither cffDNA nor the discovery of cffDNA in maternal plasma or serum is patentable, because the presence of cffDNA in maternal plasma or serum is a natural phenomenon. Docket No. 219 at 1-2; Docket No. 223 at 1, 8; *see Myriad*, 133 S. Ct. at 2116; *Prometheus*, 132 S. Ct. at 1293; *see also Funk Bros.*, 333 U.S. at 130 ("He who discovers a hitherto unknown phenomenon of nature has no claim to a monopoly of it which the law recognizes."). This is true even if the discovery of cffDNA in maternal plasma or serum was considered groundbreaking, innovative, and brilliant. *See Myriad*, 133 S. Ct. at 2117. However, the '540 patent does not claim as an invention the discovery of cffDNA in maternal plasma or serum. The '540 patent claims methods of detecting paternally inherited cffDNA in maternal plasma or serum. *See* '540 Patent at 2:1-5, 23:60-26:40. Therefore, the issue before the Court is whether the steps of the claimed methods in the '540 patent, applied to that natural phenomenon, are sufficient to render the claims patentable. *See Prometheus*, 132 S. Ct. at 1297 ("[D]o the patent claims add enough to their statements of the correlations to allow the processes they describe to qualify as patent eligible processes that apply natural laws").

A process or method is not unpatentable simply because it contains a law of nature, a natural phenomenon, or an abstract idea. *Prometheus*, 132 S. Ct. at 1293; *Flook*, 437 U.S. at 590. But, to be

patentable, a process that focuses upon the use of a natural law, a natural phenomenon, or an abstract idea must contain other elements or a combination of elements, sometimes referred to as an "inventive concept," sufficient to ensure that the patent in practice amounts to significantly more than a patent upon the natural law, natural phenomenon, or abstract idea itself. *Prometheus*, 132 S. Ct. at 1294; *see also Flook*, 437 U.S. at 594 ("[T]he discovery of such a phenomenon cannot support a patent unless there is some other inventive concept in its application."). In other words, the claimed process – apart from the natural law, natural phenomenon, or abstract idea – must involve more than "well-understood, routine, conventional activity," previously engaged in by those in the field. *Prometheus*, 132 S. Ct. at 1294, 1299; *see also id.* at 1300 ("[S]imply appending conventional steps, specified at a high level of generality, to laws of nature, natural phenomena, and abstract ideas cannot make those laws, phenomena, and ideas patentable."); *Myriad*, 133 S. Ct. at 2119-20 (explaining that an innovative method of manipulating a natural phenomenon – as opposed to applying a well-understood process in the field – would be patentable).

Here, Ariosa argues that the method steps contained in claims 1, 2, 4, 5, 8, 19-22, 24, and 25 of the '540 patent do not add enough to the natural phenomenon of paternally inherited cffDNA to make these claims patentable under § 101. Docket No. 219 at 10-20. Specifically, Ariosa argues that the additional limitations in the claims either apply well-understood, routine, and conventional activity to the natural phenomenon or limit the natural phenomenon to specific types of the natural phenomenon, which are also unpatentable. *See id.* The Court agrees. For example, claim 1 of the '540 patent claims a method for detecting cffDNA, comprising the following two steps: "amplifying a paternally inherited nucleic acid from the serum or plasma sample [from a pregnant female] and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample." '540 Patent at 23:64-67. Ariosa has presented the Court with evidence, including the specification and prosecution history of the '540 patent and testimony by Sequenom's own expert, Dr. Evans, stating that the amplification and detection of DNA sequences in plasma or serum was well known by 1997. Docket No. 219 at 10-14 (citing evidence); Docket No. 238 at 6-7 (citing evidence). For example, the specification of the '540 patent states that "[t]he preparation of serum of plasma from the maternal blood sample is carried out by standard techniques" and also states "[s]tandard nucleic acid amplification systems can be used." '540

Patent at 2:26-27, 2:44-45; *see also* Docket No. 219-7, Gindler Decl. Ex. 5 ¶ 7. In addition, the inventors during the prosecution history stated that any of the well-known, routine techniques for detection of DNA could be used to detect fetal DNA in maternal serum or plasma. Docket No. 219-4, Gindler Decl. Ex. 2 at 5, 7-8, 10, 12; *see also* '540 Patent at 1:38-43. Sequenom's expert Dr. Evans acknowledged that traditional DNA diagnostics, prior to the invention, commonly involved sample preparation, amplification, and detection. Docket No. 219-6, Gindler Decl. Ex. 4 at 188:5-13; *see also id.* at 150:18-151:7, 152:4-15. Dr. Evans also acknowledged that others before the inventors had amplified and detected nucleic acid in plasma or serum. *Id.* at 188:15-17; Docket No. 35, Evans Decl. ¶ 58; *see also* Docket No. 238-7, Gindler Decl. Ex. 16 at 485 ("There has been much interest in the use of DNA derived from plasma or serum for molecular diagnosis."). Sequenom does not contest that these steps and other steps in the patent[5] were well-understood, routine, and conventional activity by those in the field at the time of the invention. Indeed, in its reply brief and at oral argument, Sequenom acknowledges that the claims of the '540 patent merely apply "conventional techniques" to the newly discovered natural phenomenon of cffDNA. Docket No. 240 at 7 ("Just like Myriad's claim 21, the '540 patent's claims apply conventional techniques to use a newly-isolated natural phenomenon for diagnostic purposes."); Docket No. 253 at 19:7-10 ("The inventive concept was to take a known method and to look at [it] in a place where people were – where the Federal Circuit and all the experts agree

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[5] Dependent Claims 2 and 4 respectively add the limitations of requiring the use of the polymerase chain reaction ("PCR") and the use of a sequence specific probe. *See* '540 Patent at 24:60-61, 24:65-67. Ariosa has presented the Court with evidence that these two techniques were well-understood, routine, conventional activity engaged in by those in the field at the time of the invention. *See id.* at 2:44-45, 5:7-10, 6:42-7:10, 9:62-63, 10:5-7; Docket No. 35, Evans Decl. ¶ 42.

Dependent Claims 5, 8, 19, and 20 merely limit the natural phenomenon of paternally inherited cffDNA to specific types of that natural phenomenon, such as requiring that the cffDNA is from a Y chromosome or requiring that the cffDNA is at least a certain percentage of the total DNA. *See* '540 Patent at 25:1-3, 25:8-10, 25:39-26:3. A specific type of a natural phenomenon is still a natural phenomenon and, thus, is not patentable. *See Myriad*, 133 S. Ct. at 2116; *Prometheus*, 132 S. Ct. at 1293.

Dependent claims 21 and 22 add the limitations of fractionating the blood sample and providing a diagnosis based on the cffDNA. *See id.* at 26:4-26:16. Independent claims 24 and 25 contain – in addition to the limitations in claim 1 – limitations related to fractionating a blood sample. *See id.* at 26:20-36. Ariosa has presented the Court with evidence that fractionating blood and providing a diagnosis based on fetal DNA were well-understood, routine, conventional activity engaged in by those in the field at the time of the invention. *See id.* at 2:26-27; Docket No. 219-2, Gindler Decl. Ex. 3 at 6, Ex. 4 at 152:4-15, Ex. 5 ¶ 7.

were throwing waste away, to look there . . ."), 21:19-21 ("I don't disagree that if you go through all the elements in the claim you could put a check as either a conventional item or a natural phenomenon."), 37:20-22, 38:25-39:1 ("They used conventional tools to make it useful to other people."). Because the claimed processes at issue – apart from the natural phenomenon of paternally inherited cffDNA – involve no more than well-understood, routine, conventional activity, previously engaged in by those in the field, they are not drawn to patent eligible subject matter and are invalid under § 101. *See Prometheus*, 132 S. Ct. at 1294, 1299-1300; *Myriad*, 133 S. Ct. at 2119-20.

Sequenom argues that the claims are patentable because although cffDNA is not patentable, the use of cffDNA is patent eligible. Docket No. 223 at 7-10. The Court disagrees. The Supreme Court has never stated that any use of a natural phenomenon is patentable. To the contrary, the Supreme Court has held that "simply appending conventional steps, specified at a high level of generality, to laws of nature, natural phenomena, and abstract ideas cannot make those laws, phenomena, and ideas patentable." *Prometheus*, 132 S. Ct. at 1300. It is only an innovative or inventive use of a natural phenomenon that is afforded patent protection. *See Myriad*, 133 S. Ct. at 2119 ("Had Myriad created an innovative method of manipulating genes while searching for the BRCA1 and BRCA2 genes, it could possibly have sought a method patent."); *Flook*, 437 U.S. at 594 ("[A]n inventive application of the principle may be patented."). Sequenom attempts to argue that its patent claims an inventive method of using cffDNA. But, based on the undisputed facts before the Court, the only inventive part of the patent is that the conventional techniques of DNA detection known at the time of the invention are applied to paternally inherited cffDNA as opposed to other types of DNA. Thus, the only inventive concept contained in the patent is the discovery of cffDNA, which is not patentable.

The Court's conclusion conforms with the relevant Supreme Court case law, in particular *Flook* and *Myriad*. The patent in *Flook*, like the present patent, claimed methods that utilized an abstract idea or a natural phenomenon – a mathematical algorithm in *Flook*, paternally inherited cffDNA in the present case.[6] *See* 437 U.S. at 585. In *Flook*, as in here, the use of the abstract idea or the natural

[6] The Court recognizes that the claims in *Flook* utilized an abstract idea, while the present claims utilize a natural phenomenon. However, the Supreme Court has never drawn a distinction between natural phenomena, laws of nature, and abstract ideas in determining patent eligibility. To the contrary,

phenomenon is the only inventive feature of the claims. *See id.* at 588. In *Flook*, the Supreme Court noted "the only difference between the conventional methods of changing alarm limits and that described in respondent's application rests in the second step – the mathematical algorithm or formula." *Id.* at 585-86. Similarly, based on the undisputed facts, the only difference between the conventional methods of DNA detection and that described in the '540 patent rests in the application of the methods to paternally inherited cffDNA, a natural phenomenon. Sequenom argues that its use of cffDNA is inventive because prior to the invention, no one had started with the mother's plasma or serum to detect paternally inherited fetal DNA. Docket No. 223 at 7, 16. Even assuming this true, the same argument could be made for the claims in *Flook*. Prior to the invention in *Flook*, no one had used that particular mathematical formula to update alarm limits. Despite this, the Supreme Court held that the claims in *Flook* were not drawn to patent eligible subject matter. Thus, use of a newly discovered natural phenomenon, law of nature, or abstract idea will not render a claim patentable if the use of that natural phenomenon, law of nature or abstract idea is the only innovation contained in the patent. *See Flook*, 437 U.S. at 594 ("[T]he discovery of such a phenomenon cannot support a patent unless there is some other inventive concept in its application."); *Prometheus*, 132 S. Ct. at 1294, 1299 (requiring that claims – apart from the natural phenomenon – contain more than well-understood, routine, conventional activity); *Funk Bros.*, 333 U.S. at 131 ("[H]owever ingenious the discovery of that natural principle may have been, the application of it is hardly more than an advance in the packaging of the inoculants."). As explained in *Flook*, "the Pythagorean theorem would not have been patentable, or partially patentable, because a patent application contained a final step indicating that the formula, when solved, could be usefully applied to existing surveying techniques." 437 U.S. at 590. The Court similarly concludes that paternally inherited cffDNA is not patentable simply because the claims contain steps indicating that it may be detected using existing DNA detection methods.

Further, even though *Myriad* involved composition claims rather than method claims, that decision also supports the Court's conclusion. The claims in *Myriad* gave the patentees the exclusive

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the Supreme Court has applied its § 101 jurisprudence uniformly regardless of whether the claims at issue involved a natural phenomenon, law of nature, or abstract idea. *See, e.g., Myriad*, 133 S. Ct. 2116-20 (natural phenomenon); *Prometheus*, 132 S. Ct. at 1293-1302 (law of nature); *Bilski*, 130 S. Ct. at 3229-31 (abstract idea).

right to isolate the BRCA1 and BCRA2 genes. *See* 133 S. Ct. at 2113. Although the Supreme Court was not presented with method claims, the Court explained "[h]ad Myriad created an innovative method of manipulating genes while searching for the BRCA1 and BRCA2 genes, it could possibly have sought a method patent. But the processes used by Myriad to isolate DNA were well understood by geneticists at the time of Myriad's patents . . . ."[7] *Id.* at 2119-20. Similarly, had the inventors of the '540 patent created an innovative method of performing DNA detection while searching for paternally inherited cffDNA, such as a new method of amplification or fractionation, those claims would be patentable. But, the claims presently before the Court simply rely on processes to detect DNA that – as Sequenom concedes – were conventional techniques by those in the field at the time of the invention. Docket No. 240 at 7; Docket No. 253 at 19:7-10, 21:19-121, 37:20-22, 38:25-39:1.[8]

Sequenom cautions that the Court should not engage in a step-by-step dismantling of the claims. Docket No. 223 at 22-24 (citing *Diehr*, 450 U.S. at 188 ("In determining the eligibility of respondents' claimed process for patent protection under § 101, their claims must be considered as a whole. It is inappropriate to dissect the claims into old and new elements and then to ignore the presence of the old

[7] The Supreme Court drew this distinction even though Myriad was the first to use those well-understood processes to isolate the BRCA1 and BRCA2 genes. *See Myriad*, 133 S. Ct. at 2112-13. Therefore, *Myriad* also supports the principle that the use of a newly discovered natural phenomenon, law of nature, or abstract idea will not render a claim patentable if the use of that natural phenomenon, law of nature or abstract idea is the only innovation contained in the patent.

[8] The Court rejects Sequenom's argument that *Myriad* supports the patentability of the '540 patent's claims because the Supreme Court implicitly approved of claim 21 of Myriad's patent. *See* Docket No. 223 at 12; Docket No. 240 at 6-7. In *Myriad*, the Supreme Court endorsed the statement in Judge Bryson's Federal Circuit dissent that "'[a]s the first party with knowledge of the [BRCA1 and BRCA2] sequences, Myriad was in an excellent position to claim applications of that knowledge. Many of its unchallenged claims are limited to such applications.'" 133 S. Ct. at 2120. In his dissent, Judge Bryson cited to claim 21 as an example of such an application. However, the Supreme Court did not refer to claim 21, or any other method claims, as an example of that principle. *See id.* Moreover, although Sequenom argues that claim 21 merely applied the conventional steps of hybridizing and detecting with probes the BRCA1 gene, Docket No. 223 at 12, Sequenom has not presented this Court with any evidence showing that hybridizing and detecting a gene with probes was conventional activity at the time of that invention.

In addition, the Court rejects Sequenom's argument that *Myriad*'s holding that cDNA is patent eligible supports the patentability of the claims of the '540 patent. Docket No. 223 at 11; Docket No. 240 at 5. In *Myriad*, the Supreme Court held that cDNA was patent eligible because it was not a naturally occurring phenomenon. 133 S. Ct. at 2119. Here, Sequenom has failed to provide any evidence or argument stating that the methods claimed in the '540 patent produce a non-naturally occurring phenomenon. To the contrary, Sequenom concedes that cffDNA is a naturally occuring phenomenon. *See* Docket No. 223 at 1, 8.

elements in the analysis. This is particularly true in a process claim because a new combination of steps in a process may be patentable even though all the constituents of the combination were well known and in common use before the combination was made."); *Ultramercial*, 722 F.3d at 1344)). In evaluating the patentability of the claims, the Court has not dissected the claims into their individual limitations and then determined whether the individual elements are old or new. Rather, the Court has considered the claimed processes as a whole. The unrebutted evidence does not merely show that the individual steps of fractionation, amplification and detection were well-understood, routine, and conventional activity at the time of the invention. The evidence shows that its was well-understood, routine, and conventional activity to combine these steps to detect DNA in serum or plasma. *See* '540 Patent at 1:19-43; Docket No. 35, Evans Decl. ¶ 58; Docket No. 219-6, Gindler Decl. Ex. 4 at 188:5-13, 188:15-17; Docket No. 238-7, Gindler Decl. Ex. 16 at 485. Therefore, looking at the claimed processes as a whole, the only inventive component of the processes in the '540 patent is to apply those well-understood, routine processes to paternally inherited cffDNA, a natural phenomenon.

In addition, in determining whether a claim is patentable, a court should consider whether the claim poses a risk of preempting a law of nature, natural phenomenon, or abstract idea.[9] *See Accenture*, 2013 U.S. App. LEXIS 18446, at *10-11; *CLS Bank Int'l v. Alice Corp. Pty*, 717 F.3d 1269, 1280-82 (Fed. Cir. 2013) (en banc) (Lourie, J., concurring); *see also Prometheus*, 132 S. Ct. at 1294 (Supreme Court case law "warn[s] against upholding patents that claim processes that too broadly preempt the use of a natural law."); *Diehr*, 450 U.S. at 187 (noting that the claims did not preempt use of the equation). Sequenom argues that the claims of the '540 patent do not preempt all other uses of cffDNA. Docket

[9] Although the Court agrees that preemption is a consideration when performing a § 101 analysis, the Court disagrees with Sequenom that whether the claims preempt all uses of the natural phenomenon is dispositive of the analysis. *See* Docket No. 223 at 2, 20. In *Flook*, the Supreme Court held that the claims were drawn to ineligible subject matter even though the Supreme Court conceded that the claims did not wholly preempt the mathematical formula at issue. *See* 437 U.S. at 589-90. In *Bilski*, the Supreme Court held that the dependent claims at issue were drawn to ineligible subject matter even though they were limited to how the abstract idea of hedging could be used in commodities and energy markets and, thus, would not preempt use of the abstract idea in other fields. *See* 130 S. Ct. at 3231. *Flook* and *Bilski* have not been overruled and remain good precedent. *See also Ultramercial*, 722 F.3d at 1346 ("[T]he Supreme Court has stated that, even if a claim does not wholly pre-empt an abstract idea, it still will not be limited meaningfully if it contains only insignificant or token pre- or post-solution activity – such as identifying a relevant audience, a category of use, field of use, or technological environment.").

No. 223 at 20. In support of this argument, Sequenom has presented the Court with scientific articles describing methods for detecting cffDNA. Docket No. 223-1, Root Decl. Ex. A at A1875, A2011-12, A2102-05, A2273-80, Ex. F. Ariosa argues that even if these articles disclose alternative methods of detecting cffDNA, Sequenom has failed to present any evidence showing that any of these alternative methods are practical and commercially viable. Docket No. 238 at 17 n.3. In response, Sequenom argues that it is only relevant that the alternative methods can be practiced, not that they are commercially viable alternatives. Docket No. 240 at 14-15. The Court disagrees. If the alternative methods are not commercially viable, then the effect of the patent in practice would be to preempt all uses of the natural phenomenon. It is important to note that the '540 patent does not merely claim uses or applications of cffDNA, it claims methods for detecting the natural phenomenon. Because generally one must be able to find a natural phenomenon to use it and apply it, claims covering the only commercially viable way of detecting that phenomenon do carry a substantial risk of preempting all practical uses of it. It is also important to note the age of the patent. The '540 patent was issued in July 2001. That twelve years have passed since the issuance of the patent but Sequenom does not present the Court with any evidence of a commercially viable alternative method of detecting cffDNA reflects the broad scope of the '540 patent's claims and the great risk that the patent could preempt the use of cffDNA. Indeed, Sequenom itself has acknowledged the preemptive effect of its patent. *See* Docket No. 238-1, Gindler Decl Ex. 11 at 2 ("'[M]anagement believes that the in-licensed '540 patent . . . will block all non-invasive cell-free DNA-based approaches.'"), Ex. 12 at 6 ("[W]e believe [the '540 patent] is the underpinnings of this whole field, and potentially believe anybody whose [*sic*] developing, an approach that interrogates the circulating cell [free] DNA is infringing this key patent in the field.")

Further, the articles cited by Sequenom were published after the issuance of the patent and well after the date of the invention. *See* Docket No. 223-1, Root Decl. Ex. A at A2102-05 (2003), A2273-80 (2012), Ex. F (2002). Therefore, even assuming that the articles disclose alternative methods of detecting cffDNA, Sequenom has failed to show that any alternative methods existed at the time of the invention or at the time of issuance of the patent. Thus, it appears that the effect of issuing the '540 patent was to wholly preempt all known methods of detecting cffDNA at that time. Accordingly, the Court concludes that the claims at issue pose a substantial risk of preempting the natural phenomenon

of paternally inherited cffDNA and that the preemption inquiry supports the Court's conclusion that the claims are not drawn to patent eligible subject matter.

In sum, the Court concludes that, based on the undisputed facts before the Court, Ariosa has met its burden of proving by clear and convincing evidence that claims 1, 2, 4, 5, 8, 19-22, 24, and 25 of the '540 patent are not drawn to patent eligible subject matter and are invalid under 35 U.S.C. § 101.

CONCLUSION

For the foregoing reasons, the Court GRANTS Ariosa's motion for summary judgment and DENIES Sequenom's motion for summary judgment. Docket Nos. 219, 223.

IT IS SO ORDERED.

Dated: October 30, 2013

SUSAN ILLSTON
United States District Judge

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UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

ARIOSA DIAGNOSTICS, INC.,

Plaintiff,

v.

SEQUENOM, INC.,

Defendant/
Counterclaim-Plaintiff,

v.

ARIOSA DIAGNOSTICS, INC.,

Counterclaim-Defendant,

and

ISIS INNOVATION LIMITED,

Nominal Counterclaim-
Defendant.

Case No. 3:11-cv-06391-SI

[~~PROPOSED~~] FINAL JUDGMENT

Pursuant to Federal Rule of Civil Procedure 58, the Court hereby enters Final Judgment in this action as follows:

1. For the reasons stated in the Court's October 30, 2013 Order Granting Plaintiff's Motion for Summary Judgment and Denying Defendant's Motion for Summary Judgment, judgment is hereby entered in favor of Plaintiff Ariosa Diagnostics, Inc. ("Ariosa") and against Defendant Sequenom, Inc. ("Sequenom") on Sequenom's counterclaim for infringement.

2. Ariosa's claim for a declaration of noninfringement is hereby dismissed without prejudice as moot.

IT IS SO ORDERED AND ADJUDGED:

Dated: November 20, 2013

The Honorable Susan Illston
United States District Court Judge

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UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF CALIFORNIA

NATERA, INC.,

Plaintiff,

v.

SEQUENOM, INC. and ISIS INNOVATION LIMITED

Defendants.

SEQUENOM, INC.

Counterclaim-Plaintiff,

v.

NATERA, INC. and DNA DIAGNOSTICS CENTER, INC.

Counterclaim-Defendants,

and

ISIS INNOVATION LIMITED,

Nominal Counterclaim-Defendant.

Case No. 3:12-cv-00132-SI

[PROPOSED] FINAL JUDGMENT OF INVALIDITY OF ASSERTED CLAIMS OF U.S. PATENT NO. 6,258,540

Natera, Inc. ("Natera"), DNA Diagnostics Center, Inc. ("DDC"), Sequenom, Inc., and Isis Innovation, Limited ("Isis") (collectively, the "Parties"), by and through their respective counsel of record, hereby stipulate as follows:

WHEREAS Natera filed this declaratory judgment action against Sequenom and Isis seeking a declaration of non-infringement and invalidity of U.S. Patent No. 6,258,540 ("the '540 patent");

WHEREAS Sequenom counterclaimed against Natera and DDC for infringement of the '540 patent;

WHEREAS on October 30, 2013, in the related case *Ariosa Diagnostics, Inc. v. Sequenom, Inc.* (Case No. C 11-06391 SI) ("*Ariosa*"), this Court granted the motion for summary judgment made by Ariosa Diagnostics, Inc., based on this Court's determination that claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are not drawn to patent eligible matter and are invalid under 35 U.S.C. § 101, as set forth in this Court's Order Granting Plaintiff's Motion For Summary Judgment And Denying Defendant's Motion For Summary Judgment (Docket No. 254 in Case No. C 11-06391 SI) ("Summary Judgment Order");

WHEREAS Sequenom asserts two additional claims of the '540 patent (claims 13 and 18) in its counterclaim for infringement against Natera and DDC in the present case;

WHEREAS the Parties agree, without prejudice to Sequenom's right to appeal, that this Court's rationale and reasoning in its Summary Judgment Order in the *Ariosa* case that claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are invalid under 35 U.S.C. § 101 applies equally in the present case;

WHEREAS, in order to conserve judicial and party resources and allow for immediate appeal, the Parties agree that this Court should further grant summary judgment with respect to the additional claims of the '540 patent – claims 13 and 18 – asserted against Natera and DDC on the basis that the claims that were the subject of the Summary Judgment Order in the *Ariosa* case are representative of these two additional claims.

NOW, THEREFORE, IT IS STIPULATED by and among the Parties, through their respective counsel, as follows:

1. The Court's Summary Judgment Order in the *Ariosa* case applies with equal force to the present case with respect to the claims of the '540 patent asserted in both the *Ariosa* case and the present case (claims 1, 2, 5, 8, 21, 22, 24, and 25). These claims are deemed invalid under 35 U.S.C. § 101 pending appeal.

2. For purposes of patent eligibility under 35 U.S.C. § 101, claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are deemed representative of claims 13 and 18 of the '540 patent.

3. The dependent claims-in-suit in the present case (claims 13 and 18) not addressed by the Court's Summary Judgment Order in the *Ariosa* case are deemed invalid under 35 U.S.C. § 101 pending appeal, and the claims of the '540 patent addressed in the Court's Summary Judgment Order in the *Ariosa* case will be treated on appeal as representative claims of these two dependent claims.

4. Insofar as the United States Court of Appeals for the Federal Circuit (the "Federal Circuit") vacates the summary judgment as to any of claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25, any claims that depend from a claim for which summary judgment was vacated and are asserted against Natera or DDC shall be treated as revived like such claims for which summary judgment was vacated.

5. By stipulating in the present case that this Court's Summary Judgment Order in the *Ariosa* case applies with equal force to the present case with respect to the claims of the '540 patent that are asserted in both the *Ariosa* case and the present case (claims 1, 2, 5, 8, 21, 22, 24, and 25) and by treating these claims as representative of the non-overlapping dependent claims of the '540 patent in suit in the present case, Sequenom retains the right to challenge this Court's Summary Judgment Order on appeal of the present judgment (as well as appeal of judgment in the *Ariosa* case or judgment in any other related case).

6. Natera stipulates to dismissal without prejudice of its claims for declaratory judgment relating to the '540 patent that are not subject to the following judgment, and reserves its right to reinstate these claims for declaratory judgment in the present action if the Federal Circuit vacates this judgment of invalidity under 35 U.S.C. § 101.

IT IS SO STIPULATED, THROUGH COUNSEL OF RECORD.

Dated: November 18, 2013

BARTKO, ZANKEL, BUNZEL, & MILLER

By: */s/ W. Paul Schuck*
W. Paul Schuck
Attorneys for Plaintiff and Counterclaim-Defendant NATERA, INC. and Counterclaim-Defendant DNA DIAGNOSTICS CENTER, INC.

Dated: November 18, 2013

KAYE SCHOLER LLP

By: */s/ Peter E. Root*
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Attorneys for Defendants and Counterclaim Plaintiffs SEQUENOM, INC. and SEQUENOM CENTER FOR MOLECULAR MEDICINE LLC

Dated: November 18, 2013

SATTERLEE STEPHENS BURKE & BURKE LLP

By: */s/ Mario Aieta*
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Attorneys for Nominal Defendant ISIS INNOVATION LIMITED

I, Peter E. Root, am the ECF User whose ID and password are being used to file this [Proposed] Final Judgment Of Invalidity Of Asserted Claims Of U.S. Patent No. 6,258,540. In compliance with General Order 45, X.B, I hereby attest that the above counsel have concurred in this filing.

Dated: November 18, 2013

/s/ Peter E. Root
Peter E. Root

[PROPOSED] FINAL JUDGMENT OF INVALIDITY UNDER 35 U.S.C. § 101 AS TO SEQUENOM'S COUNTERCLAIM FOR INFRINGEMENT

THE COURT, having considered the foregoing stipulations of the Parties, and expressly adopting these stipulations, hereby ORDERS AND ADJUDGES:

1. Based on this Court's reasoning and rationale stated in this Court's Summary Judgment Order in the *Ariosa* case, the Court hereby enters final judgment under Rule 58 of the Federal Rules of Civil Procedure in favor of Natera as to Natera's claim for a declaratory judgment of patent invalidity pursuant to 35 U.S.C. § 101 as to claims 1, 2, 4, 5, 8, 13, 18, 19, 21, 22, 24 and 25 of the '540 Patent, which are hereby adjudged as invalid under 35 U.S.C. § 101, without prejudice to Sequenom's right to appeal;

2. Based upon this Court's finding that all '540 Patent claims asserted against Natera and DDC are invalid under 35 U.S.C. § 101, the Court hereby enters final judgment under Rule 58 of the Federal Rules of Civil Procedure in favor of Natera and DDC as to Sequenom's counterclaim for infringement of the '540 patent in the present case and Natera's declaratory judgment claims for non-infringement, without prejudice to Sequenom's right to appeal;

3. Natera's declaratory judgment claims for invalidity and non-infringement of all other claims of the '540 patent are dismissed without prejudice; and

4. All issues relating to fees and costs are reserved pending the outcome of any appeals, and the deadlines for filing such motions shall be set by the Court, upon application by the Parties, after a ruling by the United States Court of Appeals for the Federal Circuit.

IT IS SO ORDERED AND ADJUDGED:

DATED: November 27, 2013

Honorable Susan Illston
United States District Court Judge

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Attorneys for Plaintiffs and Counterclaim Defendants
Verinata Health, Inc. and The Board of Trustees of the Leland Stanford Junior University

UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

VERINATA HEALTH, INC., and THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY,

Plaintiffs,

v.

SEQUENOM, INC., and SEQUENOM CENTER FOR MOLECULAR MEDICINE, LLC,

Defendants/ Counterclaim-Plaintiffs,

v.

VERINATA HEALTH, INC., and THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY,

Counterclaim-Defendants,

and

ISIS INNOVATION LIMITED,

Nominal Counterclaim-Defendant.

Case No. 3:12-cv-00865-SI

[PROPOSED] STIPULATION AND FINAL JUDGMENT UNDER FEDERAL RULE OF CIVIL PROCEDURE 54(b)

Verinata Health, Inc. ("Verinata"), The Board of Trustees of the Leland Stanford Junior University ("Stanford"), Sequenom, Inc. and Sequenom Center for Molecular Medicine, LLC ("Sequenom"), and Isis Innovation Limited ("Isis") (collectively, the "Parties"), by and through their respective counsel of record, hereby stipulate as follows:

WHEREAS Verinata and Stanford filed suit against Sequenom in which Verinata seeks a declaratory judgment of non-infringement and invalidity of U.S. Patent No. 6,258,540 ("the '540 patent"), and Verinata and Stanford allege claims for infringement of U.S. Patent Nos. 7,888,017, 8,008,018, and 8,195,415 against Sequenom;

WHEREAS Sequenom counterclaimed against Verinata for infringement of the '540 patent;

WHEREAS, on October 30, 2013, in the related case *Ariosa Diagnostics, Inc. v. Sequenom, Inc.* (Case No. C 11-06391 SI) ("*Ariosa*"), this Court granted the motion for summary judgment made by Ariosa Diagnostics, Inc., based on this Court's determination that claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are not drawn to patent eligible matter and are invalid under 35 U.S.C. § 101, as set forth in this Court's Order Granting Plaintiff's Motion For Summary Judgment And Denying Defendant's Motion For Summary Judgment (Docket No. 254 in Case No. C 11-06391 SI) ("Summary Judgment Order");

WHEREAS Sequenom asserts six additional claims of the '540 patent (claims 6, 7, 12, 13, 15, and 18) in its counterclaim for infringement against Verinata in the present case;

WHEREAS the Parties agree, without prejudice to Sequenom's right to appeal, that this Court's rationale and reasoning in its Summary Judgment Order in the *Ariosa* case that claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are invalid under 35 U.S.C. § 101 applies equally in the present case;

WHEREAS, in order to conserve judicial and party resources and allow for immediate appeal, the Parties agree that this Court should further grant summary judgment with respect to the additional claims of the '540 patent – claims 6, 7, 12, 13, 15, and 18 – asserted against Verinata on the basis that the claims that were the subject of the Summary Judgment Order in the *Ariosa* case are representative of these six additional claims.

NOW, THEREFORE, IT IS STIPULATED by and among the Parties, through their respective counsel, as follows:

1. The Court's Summary Judgment Order in the *Ariosa* case applies with equal force to the present case with respect to the claims of the '540 patent asserted in both the *Ariosa* case and the present case (claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25). These claims are deemed invalid under 35 U.S.C. § 101 pending appeal.

2. For purposes of patent eligibility under 35 U.S.C. § 101, claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25 of the '540 patent are deemed representative of claims 6, 7, 12, 13, 15, and 18 of the '540 patent.

3. The dependent claims-in-suit in the present case (claims 6, 7, 12, 13, 15, and 18) not addressed by the Court's Summary Judgment Order in the *Ariosa* case are deemed invalid under 35 U.S.C. § 101 pending appeal, and the claims of the '540 patent addressed in the Court's Summary Judgment Order in the *Ariosa* case will be treated on appeal as representative claims of these six dependent claims.

4. Insofar as the United States Court of Appeals for the Federal Circuit (the "Federal Circuit") vacates the summary judgment as to any of claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25, any claims that depend from a claim for which summary judgment was vacated and are asserted against Verinata shall be treated as revived like such claims for which summary judgment was vacated.

5. By stipulating in the present case that this Court's Summary Judgment Order in the *Ariosa* case applies with equal force to the present case with respect to the claims of the '540 patent that are asserted in both the *Ariosa* case and the present case (claims 1, 2, 4, 5, 8, 19, 20, 21, 22, 24, and 25) and by treating these claims as representative of the non-overlapping dependent claims of the '540 patent in suit in the present case, Sequenom retains the right to challenge this Court's Summary Judgment Order on appeal of the present judgment (as well as appeal of judgment in the *Ariosa* case or judgment in any other related case).

6. Verinata stipulates to dismissal without prejudice of its claims for declaratory judgment of non-infringement and invalidity of all claims except claims 1, 2, 4, 5, 6, 7, 8, 12, 13, 15, 18, 19, 21, 22, 24 and 25 of the '540 patent.

IT IS SO STIPULATED, THROUGH COUNSEL OF RECORD.

Dated: November 18, 2013

WEIL, GOTSHAL & MANGES LLP

By: */s/ Edward R. Reines*
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Attorneys for Plaintiffs
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Dated: November 18, 2013

KAYE SCHOLER LLP

By: */s/ Peter E. Root*
Peter E. Root
Attorneys for Defendants and Counterclaim Plaintiffs SEQUENOM, INC. and SEQUENOM CENTER FOR MOLECULAR MEDICINE LLC

Dated: November 18, 2013

SATTERLEE STEPHENS BURKE & BURKE LLP

By: */s/ Mario Aieta*
Mario Aieta
Attorneys for Nominal Defendant
ISIS INNOVATION LIMITED

I, Peter E. Root, am the ECF User whose ID and password are being used to file this [Proposed] Stipulation And Final Judgment Under Federal Rule Of Civil Procedure 54(b). In compliance with General Order 45, X.B, I hereby attest that the above counsel have concurred in this filing.

Dated: November 18, 2013

/s/ Peter E. Root
Peter E. Root

[PROPOSED] FINAL JUDGMENT

THE COURT, having considered the foregoing stipulations of the Parties, and expressly adopting these stipulations, and having determined that there is no just reason for delay in entering this final judgment, hereby ORDERS AND ADJUDGES:

1. Based on this Court's reasoning and rationale stated in this Court's Summary Judgment Order in the *Ariosa* case, the Court hereby enters final judgment under Rule 54(b) of the Federal Rules of Civil Procedure in favor of Verinata as to Verinata's claim for a declaratory judgment of invalidity pursuant to 35 U.S.C. § 101 as to claims 1, 2, 4, 5, 6, 7, 8, 12, 13, 15, 18, 19, 21, 22, 24 and 25 of the '540 Patent, which are hereby adjudged as invalid under 35 U.S.C. § 101, without prejudice to Sequenom's right to appeal;

2. Based upon this Court's finding that all '540 Patent claims asserted against Verinata are invalid under 35 U.S.C. § 101, the Court hereby enters final judgment under Rule 54(b) of the Federal Rules of Civil Procedure in favor of Verinata as to Sequenom's counterclaim for infringement of the '540 patent in the present case and Verinata's declaratory judgment claims for non-infringement, without prejudice to Sequenom's right to appeal.

3. Verinata's declaratory judgment claims for invalidity and non-infringement of all other claims of the '540 patent are dismissed without prejudice; and

4. All issues relating to fees and costs are reserved pending the outcome of any appeals, and the deadlines for filing such motions shall be set by the Court, upon application by the Parties, after a ruling by the United States Court of Appeals for the Federal Circuit.

IT IS SO ORDERED AND ADJUDGED:

DATED: November 20, 2013

Honorable Susan Illston
United States District Court Judge

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US006258540B1

(12) United States Patent

Lo et al.

(10) Patent No.: US 6,258,540 B1
(45) Date of Patent: Jul. 10, 2001

(54) **NON-INVASIVE PRENATAL DIAGNOSIS**

(75) Inventors: **Yuk-Ming Dennis Lo**, Kowloon (CN); **James Stephen Wainscoat**, Oxford (GB)

(73) Assignee: **Isis Innovation Limited**, Oxford (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/380,696**

(22) PCT Filed: **Mar. 4, 1998**

(86) PCT No.: **PCT/GB98/00690**

§ 371 Date: **Nov. 29, 1999**

§ 102(e) Date: **Nov. 29, 1999**

(87) PCT Pub. No.: **WO98/39474**

PCT Pub. Date: **Sep. 11, 1998**

(30) **Foreign Application Priority Data**

Mar. 4, 1997 (GB) 9704444

(51) **Int. Cl.**[7] **C12Q 1/68**

(52) **U.S. Cl.** **435/6**; 435/91.2; 435/91.5; 435/440

(58) **Field of Search** 435/6, 91.2, 440, 435/91.5

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* cited by examiner

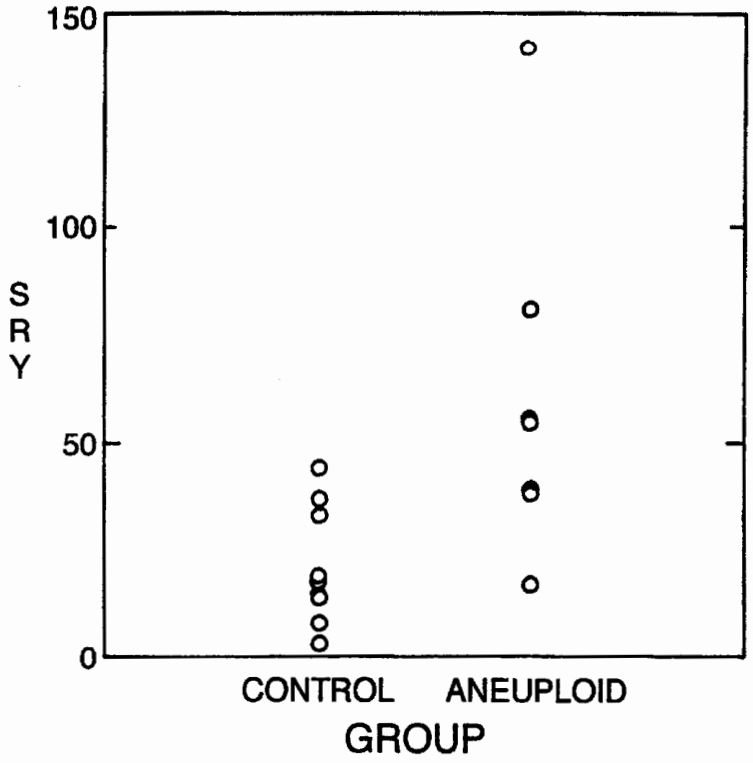
Primary Examiner—Lisa B. Arthur
Assistant Examiner—Jeanine Goldberg
(74) *Attorney, Agent, or Firm*—Volpe and Koenig, P.C.

(57) **ABSTRACT**

The invention relates to a detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a nucleic acid of foetal origin in the sample. The invention enables non-invasive prenatal diagnosis including for example sex determination, blood typing and other genotyping, and detection of pre-eclampsia in the mother.

27 Claims, 4 Drawing Sheets

Fig.1.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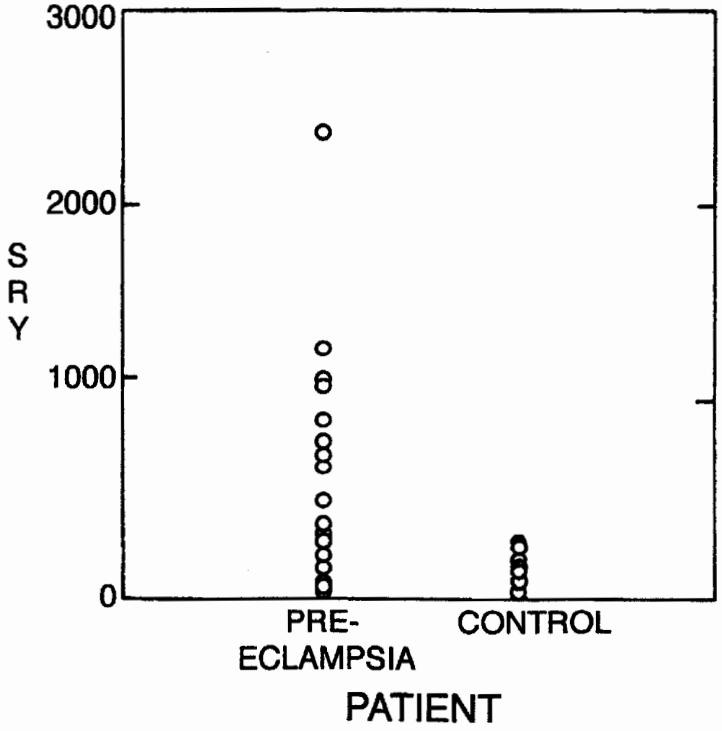
SRY
CONTROL
ANEUPLOID
GROUP

Fig.2.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1000
0
SRY
PRE-ECLAMPSIA
CONTROL
PATIENT

Fig.3A.

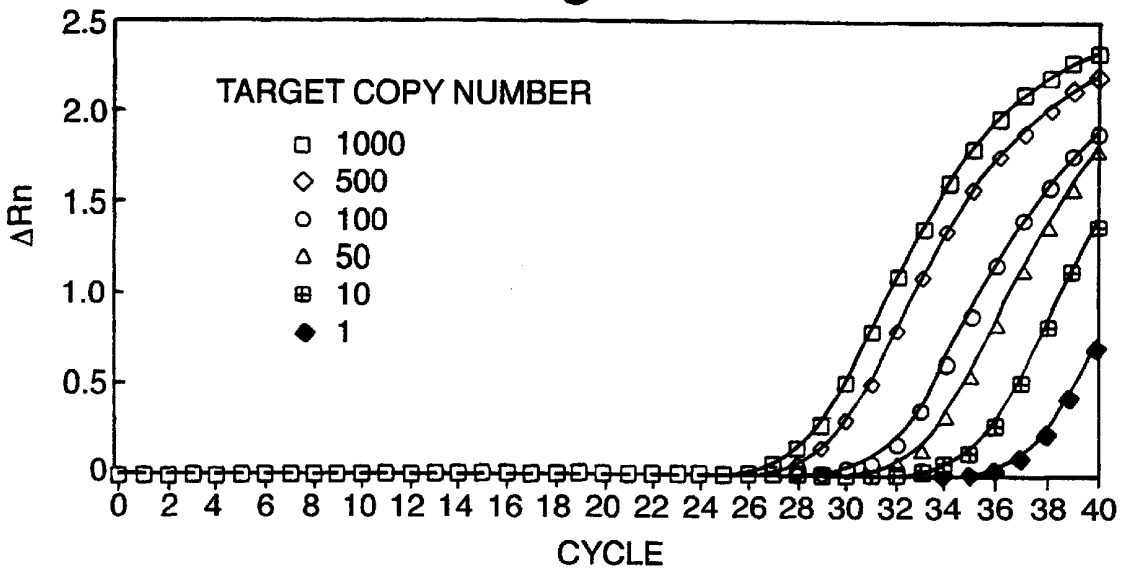
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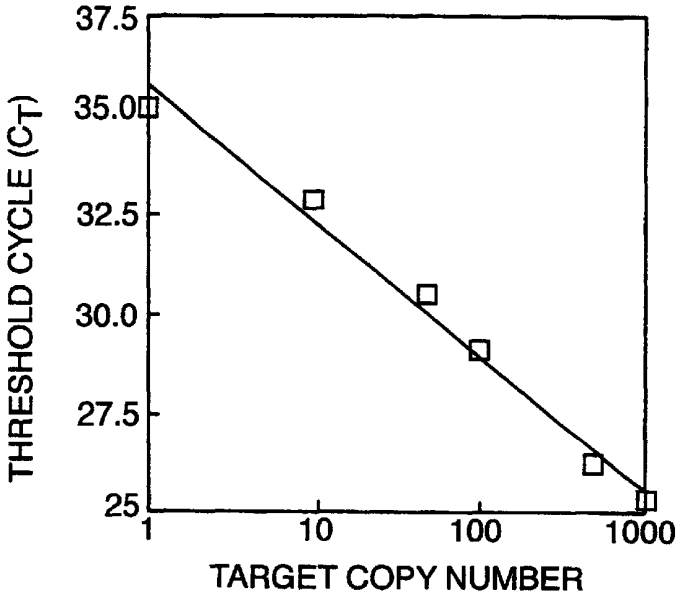
Fig.3B.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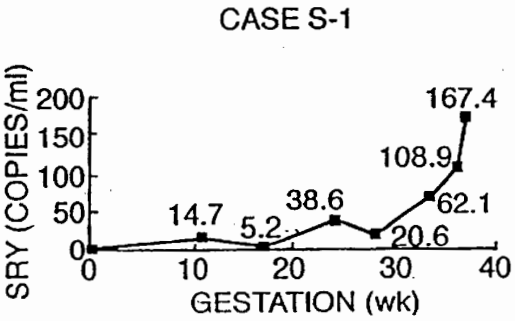

FIG. 4a

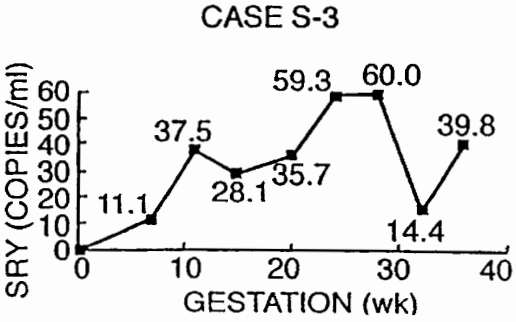

FIG. 4b

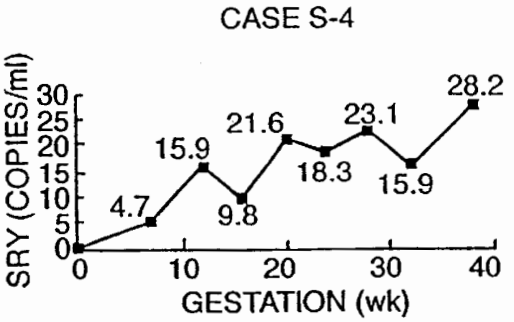

FIG. 4c

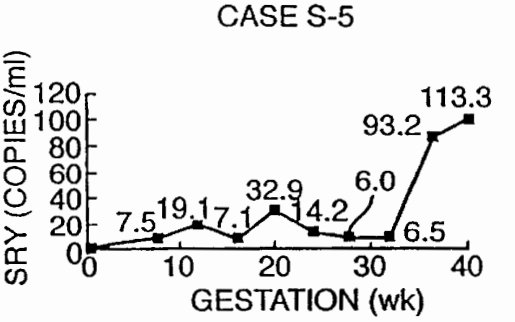

FIG. 4d

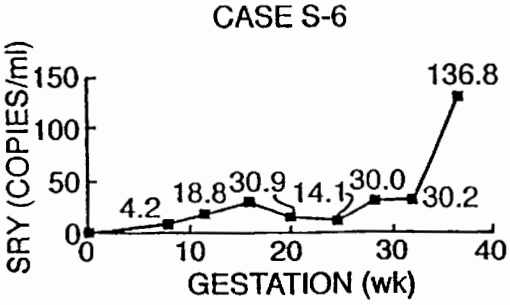

FIG. 4e

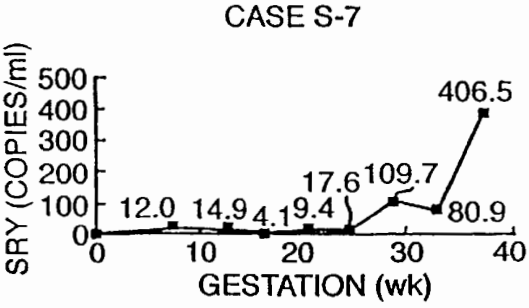

FIG. 4f

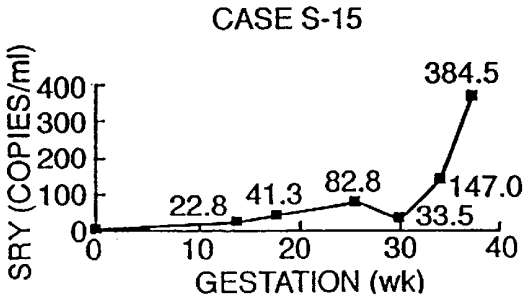

FIG. 4g

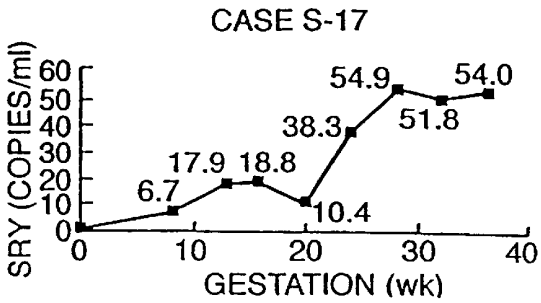

FIG. 4h

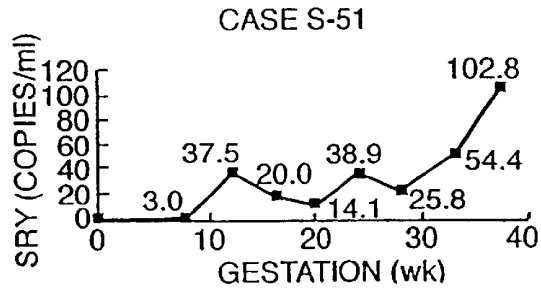

FIG. 4i

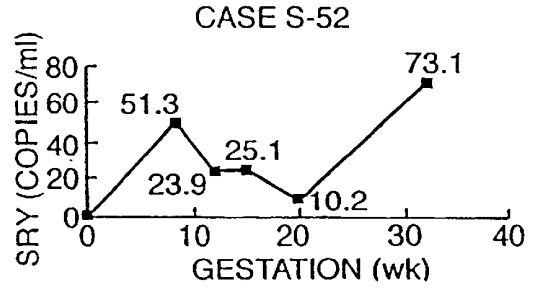

FIG. 4j

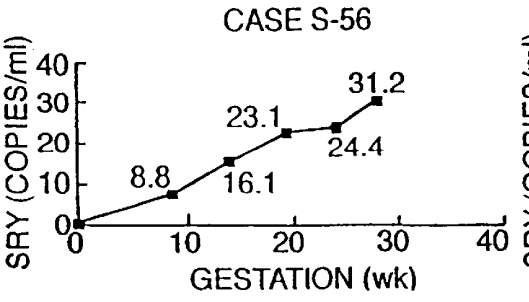

FIG. 4k

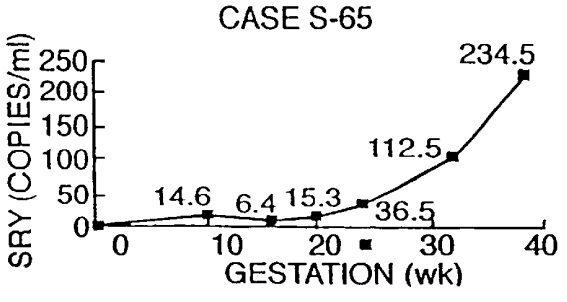

FIG. 4l

NON-INVASIVE PRENATAL DIAGNOSIS

This application is the national stage of PCT Application No. PCT/GB98/00690, filed Mar. 4, 1998 under 37 CFR 371.

This invention relates to prenatal detection methods using non-invasive techniques. In particular, it relates to prenatal diagnosis by detecting foetal nucleic acids in serum or plasma from a maternal blood sample.

BACKGROUND OF THE INVENTION

Conventional prenatal screening methods for detecting foetal abnormalities and for sex determination traditionally use foetal samples derived by invasive techniques such as amniocentesis and chorionic villus sampling. These techniques require careful handling and present a degree of risk to the mother and to the pregnancy.

More recently, techniques have been devised for predicting abnormalities in the foetus and possible complications in pregnancy, which use maternal blood or serum samples. Three markers commonly used include alpha-foetoprotein (AFP—of foetal origin), human chorionic gonadotrophin (hCG) and estriol, for screening for Down's Syndrome and neural tube defects. Maternal serum is also currently used for biochemical screening for chromosomal aneuploidies and neural tube defects. The passage of nucleated cells between the mother and foetus is now a well-recognised phenomenon (Lo et al 1989; Lo et al 1996). The use of foetal cells in maternal blood for non-invasive prenatal diagnosis (Simpson and Elias 1993) avoids the risks associated with conventional invasive techniques. WO 91/08304 describes prenatal genetic determination using foetal DNA obtained from foetal cells in the maternal blood. Considerable advances have been made in the enrichment and isolation of foetal cells for analysis (Simpson and Elias 1993; Cheung et al 1996). However, these techniques are time-consuming or require expensive equipment.

Recently, there has been interest in the use of plasma or serum-derived DNA for molecular diagnosis (Mulcahy et al 1996). In particular, it has been demonstrated that tumour DNA can be detected by the polymerase chain reaction (PCR) in the plasma or serum of some patients (Chen et al 1996; Nawroz et al 1996).

GB 2 299 166 describes non-invasive cancer diagnosis by detection of K-ras and N-ras gene mutations using PCR-based techniques.

SUMMARY AND OBJECTS OF THE INVENTION

It has now been discovered that foetal DNA is detectable in maternal serum or plasma samples. This is a surprising and unexpected finding; maternal plasma is the very material that is routinely discarded by investigators studying non-invasive prenatal diagnosis using foetal cells in maternal blood. The detection rate is much higher using serum or plasma than using nucleated blood cell DNA extracted from a comparable volume of whole blood, suggesting that there is enrichment of foetal DNA in maternal plasma and serum. In fact, the concentration of foetal DNA in maternal plasma expressed as a % of total DNA has been measured as from 0.39% (the lowest concentration measured in early pregnancy), to as high as 11.4% (in late pregnancy), compared to ratios of generally around 0.001% and up to only 0.025% for cellular fractions (Hamada et al 1993). It is important that foetal DNA is found in maternal plasma as well as serum because this indicates that the DNA is not an artefact of the clotting process.

This invention provides a detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a nucleic acid of foetal origin in the sample. The invention thus provides a method for prenatal diagnosis.

The term "prenatal diagnosis" as used herein covers determination of any maternal or foetal condition or characteristic which is related to either the foetal DNA itself or to the quantity or quality of the foetal DNA in the maternal serum or plasma. Included are sex determination, and detection of foetal abnormalities which may be for example chromosomal aneuploidies or simple mutations. Also included is detection and monitoring of pregnancy-associated conditions such as pre-eclampsia which result in higher or lower than normal amounts of foetal DNA being present in the maternal serum or plasma. The nucleic acid detected in the method according to the invention may be of a type other than DNA e.g. mRNA.

The maternal serum or plasma sample is derived from the maternal blood. As little as 10 μl of serum or plasma can be used. However it may be preferable to employ larger samples in order to increase accuracy. The volume of the sample required may be dependent upon the condition or characteristic being detected. In any case, the volume of maternal blood which needs to be taken is small.

The preparation of serum or plasma from the maternal blood sample is carried out by standard techniques. The serum or plasma is normally then subjected to a nucleic acid extraction process. Suitable methods include the methods described herein in the examples, and variations of those methods. Possible alternatives include the controlled heating method described by Frickhofen and Young (1991). Another suitable serum and plasma extraction method is proteinase K treatment followed by phenol/chloroform extraction. Serum and plasma nucleic acid extraction methods allowing the purification of DNA or RNA from larger volumes of maternal sample increase the amount of foetal nucleic acid material for analysis and thus improve the accuracy. A sequence-based enrichment method could also be used on the maternal serum or plasma to specifically enrich for foetal nucleic acid sequences.

An amplification of foetal DNA sequences in the sample is normally carried out. Standard nucleic acid amplification systems can be used, including PCR, the ligase chain reaction, nucleic acid sequence based amplification (NASBA), branched DNA methods, and so on. Preferred amplification methods involve PCR.

The method according to the invention may be particularly useful for sex determination which may be carried out by detecting the presence of a Y chromosome. It is demonstrated herein that using only 10 μl of plasma or serum a detection rate of 80% for plasma and 70% for serum can be achieved. The use of less than 1 ml of maternal plasma or serum has been shown to give a 100% accurate detection rate.

The method according to the invention can be applied to the detection of any paternally-inherited sequences which are not possessed by the mother and which may be for example genes which confer a disease phenotype in the foetus. Examples include:

a) Foetal rhesus D status determination in rhesus negative mothers (Lo et al 1993). This is possible because rhesus D positive individuals possess the rhesus D gene which is absent in rhesus D negative individuals. Therefore, the detection of rhesus D gene sequences in the plasma and serum of a rhesus D negative mother is indicative

of the presence of a rhesus D positive foetus. This approach may also be applied to the detection of foetal rhesus D mRNA in maternal plasma and serum.

b) Haemoglobinopathies (Camaschella et al 1990). Over 450 different mutations in the beta-globin gene have been known to cause beta-thalassaemia. Provided that the father and mother carry different mutations, the paternal mutation can be used as an amplification target on maternal plasma and serum, so as to assess the risk that the foetus may be affected.

c) Paternally-inherited DNA polymorphisms or mutations. Paternally-inherited DNA polymorphisms or mutations present on either a Y or a non-Y chromosome, can be detected in maternal plasma and serum to assess the risk of the foetus being affected by a particular disease by linkage analysis. Furthermore, this type of analysis can also be used to ascertain the presence of foetal nucleic acid in a particular maternal plasma or serum sample, prior to diagnostic analysis such as sex determination. This application will require the prior genotyping of the father and mother using a panel of polymorphic markers and then an allele for detection will be chosen which is present in the father, but is absent in the mother.

The plasma or serum-based non-invasive prenatal diagnosis method according to the invention can be applied to screening for Down's Syndrome and other chromosomal aneuploidies. Two possible ways in which this might be done are as follows:

a) It has been found that in pregnancy involving foetuses with chromosomal aneuploidies e.g. Down's Syndrome, the level of foetal cells circulating in maternal blood is higher than in pregnancies involving normal foetuses (Bianchi et al 1996). Following the surprising discovery disclosed herein that foetal DNA is present in maternal plasma and serum, it has also been demonstrated that the level of foetal DNA in maternal plasma and serum is higher in pregnancies where the foetus has a chromosomal aneuploidy than in normal pregnancies. Quantitative detection of foetal nucleic acid in the maternal plasma or serum e.g. a quantitative PCR assay, can be used to screen pregnant women for chromosomal aneuploidies.

b) A second method involves the quantitation of foetal DNA markers on different chromosomes. For example, for a foetus affected by Down's Syndrome the absolute quantity of foetal chromosomal 21-derived DNA will always be greater than that from the other chromosomes. The recent development of very accurate quantitative PCR techniques, such as real time quantitative PCR (Heid et al 1996) facilitates this type of analysis.

Another application of the accurate quantitation of foetal nucleic acid levels in the maternal serum or plasma is in the molecular monitoring of certain placental pathologies, such as pre-eclampsia. The concentration of foetal DNA in maternal serum and plasma is elevated in pre-eclampsia. This is probably due to the placental damage which occurs.

It is anticipated that it will be possible to incorporate the nucleic acid-based diagnosis methods described herein into existing prenatal screening programmes. Sex determination has successfully been performed on pregnancies from 7 to 40 weeks of gestation.

BRIEF DESCRIPTION OF THE DRAWINGS

In the attached figures:

FIG. **1** shows increased foetal DNA in aneuploid pregnancies compared to control pregnancies;

FIG. **2** shows increased foetal DNA in pre-eclampsia compared to control pregnancies;

FIGS. **3**A and **3**B show an amplification curve and threshold cycle for real time quantitative PCR;

FIGS. **4***a*–**4***l* show foetal DNA concentrations in maternal samples for a number of subjects at different stages of gestation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention will now be illustrated in the following Examples, which do not in any way limit the scope of the invention.

EXAMPLES

Example 1

Analysis of foetal DNA for sex determination

Patients

Pregnant women attending the Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford were recruited prior to amniocentesis or delivery. Ethics approval of the project was obtained from the Central Oxfordshire Research Ethics Committee. Informed consent was sought in each case. Five to ten ml of maternal peripheral blood was collected into an EDTA and a plain tube. For women undergoing amniocentesis, maternal blood was always collected prior to the procedure and 10 ml of amniotic fluid was also collected for foetal sex determination. For women recruited just prior to delivery, foetal sex was noted at the time of delivery. Control blood samples were also obtained from 10 non-pregnant female subjects and further sample processing was as for specimens obtained from pregnant individuals.

Sample preparation

Maternal blood samples were processed between 1 to 3 hours following venesection. Blood samples were centrifuged at 3000 g and plasma and serum were carefully removed from the EDTA-containing and plain tubes, respectively, and transferred into plain polypropylene tubes. Great care was taken to ensure that the buffy coat or the blood clot was undisturbed when plasma or serum samples, respectively, were removed. Following removal of the plasma samples, the red cell pellet and buffy coat were saved for DNA extraction using a Nucleon DNA extraction kit (Scotlabs, Strathclyde, Scotland, U.K.). The plasma and serum samples were then subjected to a second centrifugation at 3000 g and the recentrifuged plasma and serum samples were collected into fresh polypropylene tubes. The samples were stored at −20° C. until further processing.

DNA extraction from plasma and serum samples

Plasma and serum samples were processed for PCR using a modification of the method of Emanuel and Pestka (1993). In brief, 200 μl of plasma or serum was put into a 0.5 ml eppendorf tube. The sample was then heated at 99° C. for 5 minutes on a heat block. The heated sample was then centrifuged at maximum speed using a microcentrifuge. The clear supernatant was then collected and 10 μl was used for PCR.

DNA extraction from amniotic fluid

The amniotic fluid samples were processed for PCR using the method of Rebello et al (1991). One hundred μl of amniotic fluid was transferred into a 0.5 ml eppendorf tube and mixed with an equal volume of 10% Chelex-100 (Bio-Rad). Following the addition of 20 μl of mineral oil to

prevent evaporation, the tube was incubated at 56° C. for 30 minutes on a heat block. Then, the tube was vortexed briefly and incubated at 99° C. for 20 minutes. The treated amniotic fluid was stored at 4° C. until PCR and 10 μl was used in a 100 μl reaction.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was carried out essentially as described (Saiki et al 1988) using reagents obtained from a GeneAmp DNA Amplification Kit (Perkin Elmer, Foster City, Calif., USA). The detection of Y-specific foetal sequence from maternal plasma, serum and cellular DNA was carried out as described using primers Y1.7 and Y1.8, designed to amplify a single copy Y sequence (DYS14) (Lo et al 1990). The sequence of Y1.7 is 5' CAT CCA GAG CGT CCC TGG CTT 3' [SEQ ID NO: 1] and that of Y1.8 is 5° CTT TCC ACA GCC ACA TTT GTC 3' [SEQ ID NO: 2]. The Y-specific product was 198 bp. Sixty cycles of Hot Start PCR using Ampliwax technology were used on 10 μl of maternal plasma or serum or 100 ng of maternal nucleated blood cell DNA (denaturation step of 94° C. 1 minute and a combined reannealing/extension step of 57° C. 1 minute). Forty cycles were used for amplification of amniotic fluid. PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining. PCR results were scored before the foetal sex was revealed to the investigator.

Results

Sensitivity of PCR assay

Serial dilutions of male genomic DNA in 1 μg of female genomic DNA were performed and amplified by the Y-PCR system using 60 cycles of amplification. Positive signals were detected up to the 100,000 dilution, i.e., approximately the equivalent of a single male cell.

Amplification of foetal DNA sequence from maternal plasma and serum

Maternal plasma and serum samples were collected from 43 pregnant women with gestational ages from 12 to 40 weeks. There were 30 male foetuses and 13 female foetuses. Of the 30 women bearing male foetuses, Y-positive signals were detected in 24 plasma samples and 21 serum samples, when 10 μl of the respective samples was used for PCR. When nucleated blood cell DNA was used for Y-PCR, positive signals were only detected in 5 of the 30 cases. None of the 13 women bearing female foetuses and none of the 10 non-pregnant female controls resulted in a positive Y signal when either plasma, serum or cellular DNA was amplified. Accuracy of this technique, even with serum/plasma samples of only 10 μl, is thus very high and most importantly it is high enough to be useful. It will be evident that accuracy can be improved to 100% or close to 100%, for example by using a larger volume of serum or plasma.

Example 2

Quantitative analysis of foetal DNA in maternal serum in aneuploid pregnancies

The prenatal screening and diagnosis of foetal chromosomal aneuploidies is an important part of modern obstetrical care. Due to the risks associated with invasive procedures such as amniocentesis and the impracticability of performing screening with invasive methods, much effort has been devoted to the development of non-invasive screening methods for foetal chromosomal aneuploidies. The two main non-invasive methods which have been developed are maternal serum biochemical screening and ultrasound examination for nuchal translucency. These methods are both associated with significant false-positive and false-negative rates.

The demonstration of foetal nucleated cells in maternal circulation offers a new source of foetal material for the non-invasive diagnosis of foetal chromosomal aneuploidies (Simpson et al 1993). By the use of foetal nucleated cell enrichment protocols, several groups have reported the detection of aneuploid foetal nucleated cells isolated from maternal blood (Elias et al 1992; Bianchi et al 1992). Recently, it has been demonstrated that there is increased foetal nucleated cell number in maternal circulation when the foetus is suffering from a chromosomal aneuploidy (Bianchi et al 1997).

Patients samples

Blood samples from pregnant women undergoing prenatal testing were collected prior to any invasive procedure. The foetal karyotype was confirmed by cytogenetic analysis of amniotic fluid or chorionic villus samples. Approval was obtained from the Research Ethics Committee of The Chinese University of Hong Kong. Blood samples were collected into plain tubes. Following blood clotting, the samples were centrifuged at 3000 g, and serum were carefully removed and transferred into plain polypropylene tubes. The samples were stored at −70° C. or −20° C. until further processing.

DNA extraction from plasma and serum samples

DNA from serum samples were extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany) using the "blood and body fluid protocol" as recommended by the manufacturer (Chen et al 1996). Four hundred to 800 μl of plasma/serum sample was used for DNA extraction per column. The exact amount used was documented to enable the calculation of target DNA concentration.

Real time quantitative PCR

Theoretical and practical aspects of real time quantitative PCR were previously described by Heid et al (1996). Real time quantitative PCR analysis was performed using a PE Applied Biosystems 7700 Sequence Detector (Foster City, Calif., U.S.A.) which is essentially a combined thermal cycler/fluorescence detector with the ability to monitor the progress of individual PCR reactions optically. The amplification and product reporting system used is based on the 5' nuclease assay (Holland et al 1991) (the TaqMan assay as marketed by Perkin-Elmer). In this system, apart from the two amplification primers as in conventional PCR, a dual labeled fluorogenic hybridisation probe is also included (Lee et al 1993; Livak et al 1995). One fluorescent dye serves as a reporter (FAM, i.e., 6-carboxyfluorescein) and its emission spectra is quenched by a second fluorescent dye (TAMRA, i.e., 6-carboxy-tetramethylrhodamine). During the extension phase of PCR, the 5' to 3'-exonuclease activity of the Taq DNA polymerase cleaves the reporter from the probe thus releasing it from the quencher, resulting in an increase in fluorescent emission at 518 nm. The PE Applied Biosystems 7700 Sequence Detector is able to measure the fluorescent spectra of the 96 amplification wells continuously during DNA amplification and the data are captured onto a Macintosh computer (Apple Computer, Cupertino, Calif., U.S.A.).

The SRY TaqMan system consisted of the amplification primers SRY-109F, 5'-TGG CGA TTA AGT CAA ATT CGC-3' [SEQ ID NO:3]; SRY-245R, 5'-CCC CCT AGT ACC CTG ACA ATG TAT T-3' [SEQ ID NO:4]; and a dual labeled fluorescent TaqMan probe SRY-142T, 5'-(FAM) AGC AGT AGA GCA GTC AGG GAG GCA GA(TAMRA)-3' [SEQ ID NO: 5]. Primer/probe combinations were designed using the Primer Express software

(Perkin-Elmer, Foster City, Calif., U.S.A.). Sequence data for the SRY gene were obtained from the GenBank Sequence Database (accession number L08063).

TaqMan amplification reactions were set up in a reaction volume of 50 μl using components (except TaqMan probe and amplification primers) supplied in a TaqMan PCR Core Reagent Kit (Perkin-Elmer, Foster City, Calif., U.S.A.). The SRY TaqMan probe were custom-synthesised by PE Applied Biosystems. PCR primers were synthesised by Life Technologies (Gaithersburg, Md., U.S.A.). Each reaction contained 5 μl of 10× buffer A, 300 nM of each amplification primers, 100 nM of the SRY TaqMan probe, 4 mM $MgCl_2$, 200 μM each of dATP, dCTP and dGTP, 400 μM dUTP, 1.25 units of AmpliTaq Gold and 0.5 unit AmpErase uracil N-glycosylase. Five to ten μl of the extracted serum DNA was used for amplification. The exact amount used was recorded for subsequent concentration calculation. DNA amplifications were carried out in 96-well reaction plates that were frosted by the manufacturer to prevent light reflection and were closed using caps designed to prevent light scattering (Perkin-Elmer, Foster City, Calif., U.S.A.). Each sample was analysed in duplicate. A calibration curve was run in parallel and in duplicate with each analysis. The conversion factor of 6.6 pg of DNA per cell was used for expressing the results as copy numbers.

Thermal cycling was initiated with a 2-minute incubation at 50° C. for the uracil N-glycosylase to act, followed by a first denaturation step of 10 minutes at 95° C. Then, 40 cycles of 95° C. for 15 s and 60° C. for 1 minute were carried out.

Amplification data collected by the 7700 Sequence Detector and stored in the Macintosh computer were then analysed using the Sequence Detection System (SDS) software developed by PE Applied Biosystems. The mean quantity of each duplicate was used for further concentration calculation. The concentration expressed in copies/ml was calculated using the following equation:

$$C=Q\times V_{DNA}/V_{PCR}\times 1/V_{ext}$$

where

C=target concentration in plasma or serum (copies/ml);

Q=target quantity (copies) determined by sequence detector in a PCR;

V_{DNA}=total volume of DNA obtained following extraction, typically 50 μl per Qiagen extraction;

V_{PCR}=volume of DNA solution used for PCR, typically 5–10 μl

V_{ext}=volume of plasma/serum extracted, typically 400–800 μl

Anti-contamination measures

Strict precautions against PCR contamination were used (Kwok et al 1989). Aerosol-resistant pipette tips were used for all liquid handling. Separate areas were used for the setting up of amplification reactions, the addition of DNA template and the carrying out of amplification reactions. The 7700 Sequence Detector offered an extra level of protection in that its optical detection system obviated the need to reopen the reaction tubes following the completion of the amplification reactions, thus minimising the possibility of carryover contamination. In addition, the TaqMan assay also included a further level of anti-contamination measure in the form of pre-amplification treatment using uracil N-glycosylase which destroyed uracil containing PCR products (Longo et al 1990). Multiple negative water blanks were included in every analysis.

Results

Development of real time quantitative PCR

To determine the dynamic range of real time quantitative PCR, serial dilutions of male DNA were made in water consisting of the DNA equivalent from 1,000 cells to 1 cell and subjected to analysis by the SRY TaqMan system. The fewer the number of target molecules, the more amplification cycles were needed to produce a certain quantity of reporter molecules. The system was sensitive enough to detect the DNA equivalent from a single target cell.

A parameter, termed the threshold cycle (C_T) could be defined which was set at 10 standard deviations above the mean base-line fluorescence calculated from cycles 1 to 15 and was proportional to the starting target copy number used for amplification (Heid et al 1996). A plot of the threshold cycle (C_T) against the input target quantity, with the latter plotted on a common log scale, demonstrated the large dynamic range and accuracy of real time quantitative PCR.

The real time quantitative SRY system was insensitive to the existence of background female DNA from 0 to 12,800 female genome-equivalents. This greatly simplified the application of this system as separate calibration curves did not have to be constructed for different cases due to the presence of different concentrations of foetal and maternal DNA.

Quantitative analysis of foetal SRY gene from maternal serum from aneuploid and control pregnancies

Real time quantitative SRY PCR was carried out for serum DNA extracted from women bearing aneuploid and normal foetuses. Data from individual cases are plotted in FIG. **1**. Foetal DNA concentration was higher in aneuploid than control pregnancies (Mann-Whitney U Test, p=0.006).

Discussion

In this study we demonstrate that the concentration of foetal DNA in maternal serum is elevated in aneuploid pregnancies. These results indicate that foetal DNA quantitation has the potential to be used as a new screening marker for foetal chromosomal aneuploidies. A large scale population-based study could be carried out to develop cutoff values for screening purposes. It would also be useful to investigate the correlation of foetal DNA concentration with the other biochemical markers for maternal serum biochemical screening.

The mechanism(s) by which increased amounts of foetal DNA is liberated into maternal circulation in aneuploid pregnancies require further research. One possibility is related to the increased numbers of foetal nucleated cells which are released into the maternal blood in aneuploid pregnancies (Bianchi et al 1997). Another possible mechanism may be increased cell death or turnover which may be associated with chromosomal aneuploidies.

Example 3

Non-invasive prenatal determination of foetal RhD status from plasma of RhD-negative pregnant women

Introduction

The rhesus blood group system is important in transfusion and clinical medicine, being involved in haemolytic disease of the newborn, transfusion reactions and autoimmune haemolytic anaemia. Despite the widespread use of rhesus immunoglobulin prophylaxis in rhesus D (RhD)-negative mothers, rhesus isoimmunisation still occurs. In those cases where the father is heterozygous for RhD gene, there is a 50% chance that the foetus is RhD-positive and 50% chance that the foetus is RhD-negative. The prenatal determination of foetal RhD status in these cases is clinically useful because no further prenatal invasive testing or therapeutic manoeuvres are necessary if the foetus can be shown to be RhD-negative.

Advances towards this goal have been made possible recently through the cloning of the human RhD gene (Le Van Kim et al 1992) and the demonstration that RhD-negative individuals lack the RhD gene (Colin et al 1991). Prenatal determination of foetal RhD status has been performed using PCR-based techniques on amniotic fluid samples (Bennett et al 1993).

A number of groups have also investigated the possibility of using foetal cells in maternal blood for the determination of foetal RhD status (Lo et al 1993). The main problem with this approach is that the system is not sufficiently reliable without foetal cell enrichment or isolation procedure as demonstrated by the high false-positive and false-negative rates on unenriched samples. Foetal cell enrichment or isolation procedures, on the other hand, are tedious and expensive to perform (Geifman-Holtzman et al 1996; Sekizawa et al 1996).

Our discovery of the presence of foetal DNA in maternal plasma and serum offers a new approach for non-invasive prenatal diagnosis.

Materials and Methods

Patients

Pregnant women attending the Nuffield Department of Obstetrics & Gynaecology were recruited with informed consent. Approval of the project was obtained from the Central Oxfordshire Research Ethics Committee. Women in the second trimester of pregnancy were recruited just prior to amniocentesis. Blood samples were collected prior to any invasive procedures. Ten ml of amniotic fluid was also collected for foetal RhD genotyping. Women in the third trimester of pregnancy were recruited just prior to delivery. A sample of cord blood was taken following delivery for the ascertainment of foetal RhD status by serological methods.

Sample preparation

Blood samples were collected into tubes containing EDTA. The samples were centrifuged at 3000 g, and plasma was carefully removed and transferred into plain polypropylene tubes. Great care was taken to ensure that the buffy coat was not disturbed. The buffy coat samples were stored at −20° C. until further processing. The plasma samples were then recentrifuged at 3000 g and plasma was again carefully removed and transferred into a fresh set of plain polypropylene tubes. The samples were stored at −20° C. until further processing.

DNA extraction from plasma and serum samples

DNA from plasma and buffy coat samples were extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany) using the "blood and body fluid protocol" as recommended by the manufacturer (Cher et al 1996). Eight hundred μl of plasma sample and 200 μl of buffy coat sample was used for DNA extraction per column.

Real time quantitative PCR

Real time quantitative PCR analysis was performed as described in Example 2 with the following modifications.

The RhD TaqMan system consisted of the amplification primers RD-A: 5'-CCT CTC ACT GTT GCC TGC ATT-3' [SEQ ID NO: 6]; RD-B: 5'-AGT GCC TGC GCG AAC ATT-3' [SEQ ID NO: 7); and a dual labelled fluorescent TaqMan probe RD-T,5'-(FAM)TAC GTG AGA AAC GCT CAT GAC AGC AM GTC T(TAMRA)-3' [SEQ ID NO: 8]. Primer/probe combinations were designed using the Primer Express software (Perkin-Elmer, Foster City, Calif., U.S.A.). Sequence data for the RhD gene were as previously described (Le Van Kim et al 1992).

The beta-globin TaqMan system consisted of the amplification primers beta-globin-354F, 5'-GTG CAC CTG ACT CCT GAG GAG A-3' [SEQ ID NO: 9]; beta-globin-455R, 5'-CCT TGA TAC CM CCT GCC CAG-3'; and a dual labelled fluorescent TaqMan probe beta-globin-402T, 5'-(FAM)MG GTG AAC GTG GAT GM GTT GGT GG(TAMRA)-3' [SEQ ID NO: 10]. Primer/probe combinations were designed using the Primer Express software (Perkin-Elmer, Poster City, Calif., U.S.A.). Sequence data were obtained from the GenBank Sequence Database: accession number U01317.

Results

Development of real time TaqMan PCR

The real time sequence detector is able to measure the fluorescence intensity of the liberated reporter molecules cycle after cycle. A parameter, termed the threshold cycle (C_T), could be defined which was set at 10 standard deviations above the mean base-line fluorescence calculated from cycles 1 to 15 (Heid et al 1996). An amplification reaction in which the fluorescence intensity rises above the threshold during the course of thermal cycling is defined as a positive reaction.

To determine the sensitivity of TaqMan PCR, serial dilutions of genomic DNA isolated from a RhD-positive individual were made in water consisting of the DNA equivalent from 1,000 cells to 1 cell and subjected to analysis by the SRY TaqMan system. The fewer the number of target molecules, the more amplification cycles were needed to produce a certain quantity of reporter molecules. The system was sensitive enough to detect the DNA equivalent from a single target cell.

Correlation of serology and genotyping of the RhD-negative women

The 21 pregnant women enrolled in this study were all serologically RhD-negative. Genomic DNA (10 ng) isolated from the buffy coat from each woman was subjected to the RhD TaqMan assay and in each case a negative result was found; thus demonstrating complete correlation between the serology and genotyping.

RhD genotyping from DNA isolated from maternal plasma

DNA extracted from the plasma of the 21 RhD-negative pregnant women were subjected to the RhD TaqMan assay. There was complete correlation between the foetal RhD genotype predicted from maternal plasma analysis and the result obtained from genotyping the amniotic fluid and serological testing of the cord blood (Table 1).

As a control for the amplifiability of DNA extracted from maternal plasma, these samples were also subjected to the beta-globin TaqMan assay. In every case, a positive TaqMan signal was generated.

Discussion

In this study we have demonstrated the feasibility of performing non-invasive foetal RhD genotyping from maternal plasma. This represents the first description of single gene diagnosis from maternal plasma. Our results indicate that this form of genotyping is highly accurate and can potentially be used for clinical diagnosis. This high accuracy is probably the result of the high concentration of foetal DNA in maternal plasma.

The rhesus family of polypeptides are encoded by two related genes: the CcEe gene and the RhD gene (Le Van Kim et a/ 1992; Chérif-Zahar et al 1990). Due to the complexity of the Rh genetic systems, a number of primer sets have been described for RhD genotyping (Bennet et al 1993; Lo et al 1993; Aubin et al 1997). In order to ensure the accuracy of our genotyping system in the study samples, we performed

a control genotyping of buffy coat DNA of our patient population. In all cases there was complete correlation between serology and genotype. It is likely that for robust clinical diagnosis, multiple primer sets are preferred. The TaqMan chemistry can easily accommodate the inclusion of multiple primer/probe sets.

The correlation between the severity of foetal haemolytic disease and maternal and-D level is an area which required further investigation. It is possible that increased amount of foetal DNA is liberated into the maternal circulation in the presence of increased foetal haemolysis.

TABLE 1

RhDd genotyping from plasma from RhD-negative pregnant women

Case	Foetal RhD genotype	Maternal Plasma RhD TaqMan signal
1	−	−
2	−	−
3	−	−
4	+	+
5	+	+
6	−	−
7	−	−
8	+	+
9	+	+
10	−	−
11	+	+
12	+	+
13	+	+
14	+	+
15	−	−
16	+	+
17	+	+
18	+	+
19	+	+
20	+	+
21	+	+

Example 4

Elevation of foetal DNA concentration in maternal serum in pre-eclamptic pregnancies

Introduction

Pre-eclampsia is an important cause of maternal and foetal mortality and morbidity. Despite much research, the pathogenesis of this condition is still unclear. The disorder is mainly recognised by the concurrence of pregnancy-induced changes which regress after delivery, of which hypertension and proteinuria are the most commonly used clinical criteria. Some investigators have suggested that pre-eclampsia is the result of abnormal trophoblastic implantation, probably mediated by immunological mechanisms. Other investigators have found pathological changes in the spiral arteries in the decidua and myometrium in which partial occlusion by fibrinoid material is one feature.

In this Example we use a real time quantitative PCR assay to show the concentration of foetal DNA in the serum of women suffering from pre-eclampsia. Y chromosomal sequences from male foetuses were used as a foetal marker.

Materials and Methods

Patients

Pregnant women attending the Department of Obstetrics & Gynaecology at the Prince of Wales Hospital, Shatin, Hong Kong and the Nuffield Department of Obstetrics & Gynaecology, Oxford, U.K. were recruited with informed consent. Approval was obtained from the Research Ethics Committee of The Chinese University of Hong Kong and the Central Oxfordshire Research Ethics Committee. Pre-eclampsia was defined as a sustained rise in diastolic blood pressure to 90 mmHg or higher from previously lower values, with new and sustained proteinuria in the absence of urinary tract infection. The control pregnant women were not on medication and had no hypertension or proteinuria (defined as more than a trace on dipstick urinalysis). The pre-eclamptic and control subjects were matched for gestational age.

Sample preparation

Blood samples were collected into plain tubes. Following blood clotting, the samples were centrifuged at 3000 g, and serum were carefully removed and transferred into plain polypropylene tubes. The samples were stored at −70° C. or −20° C. until further processing.

DNA extraction from plasma and serum samples DNA from serum samples were extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany) using the "blood and body fluid protocol" as recommended by the manufacturer (Chen et al 1996). Four hundred to 800 μl of plasma/serum sample was used for DNA extraction per column. The exact amount used was documented to enable the calculation of target DNA concentration.

Real time quantitative PCR

Real time quantitative PCR analysis was performed as described in Example 2.

Results

Quantitative analysis of foetal SRYgene from maternal serum

Real time quantitative SRY PCR was carried out for serum DNA extracted from pre-eclamptic and control patients. Data from individual cases are plotted in FIG. **2**. The median foetal DNA concentrations in pre-eclamptic and control pregnancies were 381 copies/ml and 76 copies/ml, respectively. Foetal DNA concentration was higher in pre-eclamptic than control pregnancies (Mann-Whitney U Test, $p<0.0001$).

Discussion

Our data indicate that the concentration of foetal DNA is higher in pre-eclamptic compared with non-pre-eclamptic pregnancies. These results indicate that foetal DNA concentration measurement in maternal plasma may be used as a new marker for pre-eclampsia. Compared with other markers for pre-eclampsia, foetal DNA measurement is unique in that it is a genetic marker while other markers, such as activin A and inhibin A, are generally hormonal markers. By its nature, a test based on a genetic marker has the advantage that it is completely foetal-specific.

Further research will be required to investigate whether the level of foetal DNA is related to the severity of pre-eclampsia. Our discovery also opens up research into the potential application of foetal DNA quantitation to predict the occurrence of pre-eclampsia, prior to the development of clinical signs such as hypertension and proteinuria.

The mechanism by which increased amounts of foetal DNA is liberated into the circulation of pre-eclamptic women is unclear at present. Possible mechanisms include damage to the placental interface resulting in foetal cell death and the consequent release of foetal DNA into maternal circulation. A second mechanism is due to the increased trafficking of foetal cells into maternal circulation in pre-eclampsia. Foetal DNA is then liberated following their destruction in the maternal circulation. Future studies correlating the levels of foetal cells and foetal DNA would be necessary to address these issues.

Example 5

Quantitative analysis of foetal DNA in maternal plasma and serum

Introduction

We have demonstrated that foetal DNA is present in maternal plasma and serum. Detection of foetal DNA sequences was possible in 80% and 70% of cases using just 10 μl of boiled plasma and serum, respectively (Lo et al. 1997).

These observations indicate that maternal plasma/serum DNA may be a useful source of material for the non-invasive prenatal diagnosis of certain genetic disorders. To demonstrate that clinical applications are possible, a number of important questions need to be answered. First, foetal DNA in maternal plasma and serum needs to be shown to be present in sufficient quantities for reliable molecular diagnosis to be carried out. Second, data on the variation of foetal DNA in maternal plasma and serum with regard to gestation age is required to determine the applicability of this technology to early prenatal diagnosis.

In this Example we have addressed both of these issues by developing a real time quantitative TaqMan polymerase chain reaction (PCR) assay (Heid et al. 1996) for measuring the copy numbers of foetal DNA molecules in maternal plasma and serum. This technique permits continuous optical monitoring of the progress of an amplification reaction, giving accurate target quantitation over a wide concentration range. Our data show that foetal DNA is present in maternal plasma and serum at concentrations similar to those achieved by many foetal cell enrichment protocols. We have also investigated the changes of foetal DNA concentration in maternal serum at different gestational ages. Using this plasma or serum-based approach, we show that the reliable detection of foetal DNA is achievable and therefore useful for the non-invasive prenatal diagnosis of selected genetic disorders.

Subjects and Methods

Patients

Pregnant women attending the Department of Obstetrics & Gynecology at the Prince of Wales Hospital, Shatin, Hong Kong were recruited with informed consent. Approval was obtained from the Research Ethics Committee of The Chinese University of Hong Kong. For women studied at a single time point, early pregnancy samples were obtained prior to amniocentesis or chorionic villus sampling while late pregnancy samples were collected just prior to delivery. Five to ten ml of maternal peripheral blood was collected each into one tube containing EDTA and one plain tube. Subjects studied at multiple time points were recruited from the in vitro fertilization program, prior to conception. Five to ten ml of maternal blood from these subjects was collected into a plain tube at each studied time point. For women undergoing prenatal diagnosis, the sex of the baby was ascertained from cytogenetic results from the amniocentesis or chorionic villus samples. For women recruited just prior to delivery or from the in vitro fertilization program, foetal sex was noted at the time of delivery.

Sample preparation

Blood samples were centrifuged at 3000 g, and plasma and serum were carefully removed from the EDTA-containing and plain tubes, respectively, and transferred into plain polypropylene tubes. Great care was taken to ensure that the buffy coat or the blood clot was undisturbed when plasma or serum samples, respectively, were removed. The plasma and serum samples were recentrifuged at 3000 g and the supernatants were collected into fresh polypropylene tubes. The samples were stored at −20° C. until further processing.

DNA extraction from plasma and serum samples DNA from plasma and serum samples were extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany) using the "blood and body fluid protocol" as recommended by the manufacturer (Chen et al. 1996). Four hundred to 800 l of plasma/serum sample was used for DNA extraction per column. The exact amount used was documented to enable the calculation of target DNA concentration.

Real time quantitative PCR

Real time quantitative PCR analysis was performed as described in Example 2, using the SRY TaqMan system and the beta-globin TaqMan system described in the previous Examples.

Identical thermal profile was used for both the SRY and beta-globin TaqMan systems. Thermal cycling was initiated with a 2-minute incubation at 50° C. for the uracil N-glycosylase to act, followed by a first denaturation step of 10 minutes at 95° C. Then, 40 cycles of 95° C. for 15 s and 60° C. for 1 minute were carried out.

Results

Development of real time quantitative PCR

To determine the dynamic range of real time quantitative PCR, serial dilutions of male DNA were made in water consisting of the DNA equivalent from 1,000 cells to 1 cell and subjected to analysis by the SRY TaqMan system. FIG. **3**A demonstrates that the amplification curve shifted to the right as the input target quantity was reduced. This was expected as reactions with fewer target molecules required more amplification cycles to produce a certain quantity of reporter molecules than reactions with more target molecules. The system was sensitive enough to detect the DNA equivalent from a single target cell.

FIG. **3**B shows a plot of the threshold cycle (C_T) against the input target quantity, with the latter plotted on a common log scale. The C_T was set at 10 standard deviations above the mean base-line fluorescence calculated from cycles 1 to 15 and was proportional to the starting target copy number used for amplification (Heid et al. 1996). The linearity of the graph demonstrates the large dynamic range and accuracy of real time quantitative PCR. Similar results were obtained using the beta-globin TaqMan system (results not shown).

The real time quantitative SRY system was insensitive to the existence of background female DNA from 0 to 12,800 female genome-equivalents. This greatly simplified the application of this system as within this range, separate calibration curves did not have to be constructed for different cases due to the presence of different concentrations of foetal and maternal DNA.

The reproducibility of DNA extraction from plasma and serum using the Qiagen protocol was tested by performing replicate extractions (10 for each case) from plasma and serum samples from normal individuals. These replicate extractions were then subjected to real time quantitative PCR using the beta-globin system. The coefficient of variation (CV) of C_T values of these replicate extractions was 1.1%.

Quantitative analysis using the real time beta-globin TaqMan system

The concentration of beta-globin sequences in maternal plasma and serum samples was used as a measure of the total amount of extracted DNA, i.e., maternal and foetal DNA extracted from plasma and serum samples from 50 pregnant

women was analysed using the beta-globin TaqMan system. Twenty-five cases were recruited during the first and second trimesters (gestational age: 11 to 17 weeks) and were denoted as early pregnancy samples in Table 2. The other twenty-five cases were recruited just prior to delivery (gestational age: 37 to 43 weeks) and were denoted as late pregnancy samples in Table 1. The concentrations of beta-globin sequences in maternal plasma and serum are listed in Table 2. These results show that serum contains more DNA than plasma (Wilcoxon Signed Rank Test, $p<0.0005$), with a mean concentration of serum DNA 14.6 times that of plasma DNA in our studied population. The concentration of beta-globin sequences in maternal plasma from early and late pregnancy samples are compared in Table 2. These data show that the total amount of plasma DNA increases as pregnancy progresses (Mann-Whitney Rank Sum Test, $p<0.0005$).

Quantitative analysis of foetal SRY gene from maternal plasma and serum

Real time quantitative analysis using the SRY TaqMan system was carried out on DNA extracted from maternal plasma and serum to determine the amount of foetal DNA. Of the 25 early pregnancy samples (gestational age: 11 to 17 weeks), 13 were from women bearing male foetuses and 12 were from women bearing female foetuses. Of the 25 late pregnancy samples (gestational age: 37 to 43 weeks), 14 were from women bearing male foetuses and 11 were from women bearing female foetuses. A positive signal was obtained in each of the 27 women bearing male foetuses and no signal was detected in each of the 23 women bearing female foetuses. Fourteen women had a history of delivering a previous male baby and 5 of these were carrying a female baby in the current studied pregnancy.

Quantitative SRY data from the 27 women bearing male foetuses are summarised in Table 3. These data show that the concentrations of foetal DNA in plasma and serum are higher in late gestation than in early gestation (Mann-Whitney Rank Sum Test, $p<0.0005$). The mean concentrations of foetal DNA in maternal plasma and serum are 11.5 times and 11.9 times, respectively, higher in late gestation compared with early gestation. The absolute concentrations of foetal DNA in maternal plasma and serum were similar in individual cases. The fractional concentration of foetal DNA in early pregnancy ranges from 0.39% to 11.9% (mean: 3.4%) in plasma and 0.014% to 0.54% (mean: 0.13%) in serum. In late pregnancy, the fraction of foetal DNA ranges from 2.33% to 11.4% (mean: 6.2%) in plasma and 0.032% to 3.97% (mean: 1.0%) in serum.

Sequential follow up of women who conceived by in vitro fertilization

Twenty women who conceived by in vitro fertilization (IVF) were followed up at pre-conception and at multiple time points during pregnancy. All twenty subjects had singleton pregnancies as determined by ultrasound scanning. Twelve women delivered male babies and the remaining 8 delivered female babies. None of the women carrying male foetuses had a history of pregnancy-associated complications. Subject S-51 (FIG. **4**) underwent chorionic villus sampling at 12 weeks. Subjects S-1 and S-56 (FIG. **4**) had amniocentesis at 16 and 17 weeks, respectively. A total of 163 serum samples from these 20 women were analysed using the real time quantitative SRY TaqMan system. None of the 65 serum samples from the 8 women bearing female babies gave a positive SRY signal. The concentrations of foetal DNA in the 98 serum samples from women carrying male babies are plotted in FIGS. **4***a*–**4***l*.

Discussion

We have developed an accurate real time quantitative PCR system for determining the concentration of foetal DNA in maternal plasma and serum. This system has a number of advantages: (1) a large dynamic range of over 5 orders of magnitude (Heid et al. 1996); (2) a high throughput and fast turnaround time—96 samples could be simultaneously amplified and quantified in approximately 2 hours; and (3) the use of a homogeneous amplification/detection system which requires no post-PCR processing and therefore minimizes the risk of carryover contamination.

The most important observation in this study is the very high concentration of foetal DNA in maternal plasma and serum. Bianchi et al reported that the average number of foetal cells in maternal blood in normal pregnancies was 19 in 16 ml of maternal blood, i.e., 1.2 cells/ml during the second trimester (Bianchi et al. 1997). Therefore, the mean concentration of foetal DNA in maternal plasma and serum is 21.2 (25.4/1.2) and 23.9 (28.7/1.2) times, respectively, higher than that in the cellular fraction of maternal blood at the same gestation. The relative concentration of foetal to total plasma DNA is even higher. Thus, in early pregnancy, foetal DNA in maternal plasma constitutes a mean of 3.4% of the total plasma DNA. The respective figure in late pregnancy is 6.2%. Hamada et al reported that the frequency of foetal cells in the second trimester was 0.0035% while that in the third trimester was 0.008% (Hamada et a/. 1993). The fetomaternal ratio is, therefore, 97Sfold and 775-fold higher in maternal plasma than in the cellular fraction at the respective gestational age. Indeed, the fetomaternal ratio in plasma DNA is comparable to that following many foetal cell enrichment protocols. For example, Bianchi et al reported that following foetal nucleated red cell enrichment using fluorescence activated cell sorting, the resulting foetal cells constituted 0.001%–5% of the sorted cell populations as determined by quantitative PCR analysis (Bianchi et al. 1994). In a similar study using cell sorting and foetal cell detection using fluorescence in situ hybridization, Sohda et al found that on average 4.6% of the sorted cells were of foetal origin (Sohda et al. 1997). Maternal plasma, therefore, offers an easily accessible foetal DNA source for prenatal genetic analysis.

We have demonstrated that the absolute concentration of foetal DNA in maternal plasma is similar to that in maternal serum. The main difference lies in the presence of a larger quantity of background maternal DNA in serum compared with plasma, possibly due to the liberation of DNA during the clotting process. While this exerts no noticeable effect on the efficiency of foetal DNA detection using the real time TaqMan system, it is possible that with the use of less sensitive methods, e.g., conventional PCR followed by ethidium stained agarose gel electrophoresis, maternal plasma may be preferable to maternal serum for robust foetal DNA detection.

The high concentration of foetal DNA in maternal plasma and serum has allowed us to reliably detect the presence of foetal genetic material. Of the 263 serum or plasma samples analysed in this study, we were able to detect foetal SRY gene in maternal plasma or serum from every subject who was carrying a male baby at the time of venesection. This robust detection rate was obtained using DNA extracted from just 40–80 μl of maternal plasma and serum. This volume represents a 4–8 fold increase over the 10 μl of boiled maternal plasma or serum reported in our previous study (Lo et al. 1997) and results in significant improvement in sensitivity. The specificity was preserved as we did not observe amplification signals from samples obtained pre-

conception or from subjects carrying a female foetus. From the data obtained thus far, plasma/serum analysis did not appear to be significantly affected by the persistence of foetal cells from previous pregnancies (Bianchi et al. 1996). Thus, we did not obtain any false positive results from women who had carried a previous male baby but who were carrying a female baby at the time of blood sampling for this study.

The sequential study on patients undergoing IVF gave a number of important results. First, all of the 12 patients carrying male babies were shown to be negative for SRY sequences in their sera prior to conception. This provided convincing evidence that the SRY sequence detected by the TaqMan assay did indeed originate from the male foetus in the current pregnancy. Second, we were able to detect foetal SRY sequences as early as the 7th week of gestation; thus indicating that foetal genetic analysis in maternal plasma/serum could be used in the first trimester. Third, we showed that foetal DNA concentration increased as pregnancy progressed (FIGS. **4***a*–**4***l*). This last point was also confirmed by data obtained from women studied at a single time point. Women recruited late in pregnancy had higher foetal DNA concentrations in their plasma and serum (Table 3).

In addition to the increase in foetal DNA concentration as pregnancy progresses, our data also indicate that maternal plasma DNA also increases with gestation (Table 2). The biologic basis for this phenomenon is unclear at present. Possible explanations include the increase in size of the fetomaternal interface as gestation progresses and possible reduction in DNA clearance associated with other physiologic changes in pregnancy.

For selected disorders, foetal genetic information could be acquired more economically and rapidly from maternal plasma or serum than by using foetal cells isolated from maternal blood. We envisage that foetal DNA analysis in maternal plasma and serum would be most useful in situations where the determination of foetal-derived paternally-inherited polymorphisms/mutations or genes would be helpful in clinical prenatal diagnosis (Lo et al. 1994). Examples include foetal sex determination for the prenatal diagnosis of sex-linked disorders, foetal rhesus D status determination in sensitized rhesus negative pregnant women (Lo et al. 1993), autosomal dominant disorders in which the father carries the mutation and autosomal recessive genetic disorders in which the father and mother carry different mutations (Lo et al. 1994), e.g., certain hemoglobinopathies (Camaschella et al. 1990) and cystic fibrosis. Due to the much reduced maternal background and high foetal DNA concentration in maternal plasma and serum, we predict that this type of analysis would be much more robust compared with their application for detecting unsorted foetal cells in maternal blood. The ability for allelic discrimination (Lee et al. 1993; Livak et al. 1995) allows the homogeneous TaqMan assay to be used for this purpose. The high throughput and anti-contamination capability of this system makes it an attractive candidate for large scale clinical application.

Bianchi et al recently reported that foetal cells in maternal blood were increased in aneuploid pregnancies (Bianchi et al. 1997) and it has been demonstrated (Example 2) that the foetal DNA concentration in maternal plasma and serum is also elevated in these pregnancies. This provides a new screening test for foetal chromosomal disorders. For this application, foetal DNA quantitation systems can be developed for polymorphic markers outside the Y chromosome so that quantitation can be applied to female foetuses. Autosomal polymorphic systems which may be used for this purpose have already been described (Lo et al. 1996). However, foetal cell isolation techniques would still be necessary for a definitive cytogenetic diagnosis. Similarly, foetal cell isolation would also be required for direct mutational analysis of autosomal recessive disorders caused by a single mutation. It is likely that foetal cell isolation and analysis of foetal DNA in maternal plasma/serum would be used as complementary techniques for non-invasive prenatal diagnosis.

The biologic basis by which foetal DNA is liberated into maternal plasma remains to be elucidated. It is possible that foetal DNA is released from cell lysis resulting from physical and immunologic damage, or through developmentally associated apoptosis of foetal tissues. It is also likely that increased amounts of foetal DNA may be found in conditions associated with placental damage, such as pre-eclampsia. The real time quantitative PCR system described here offers a powerful tool to study these unexplored pathophysiologic aspects of foetal DNA in maternal plasma and may improve our understanding of the fetomaternal relationship.

TABLE 2

Quantitative analysis of maternal plasma and serum using the beta-globin TaqMan assay

	Mean (copies/ml)	Median (copies/ml)	Range (copies/ml)
Plasma (Early + Late Pregnancy)	3466	1594	356–31875
Serum (Early + Late Pregnancy)	50651	34688	5813–243750
Plasma (Early Pregnancy)	986	975	356–1856
Plasma (Late Pregnancy)	5945	4313	1125–31875

TABLE 3

Quantitation of foetal DNA in maternal plasma and serum: relationship with gestational age

	SRY concentation (copies/ml)			
	Early Pregnancy		Late Pregnancy	
	Plasma	Serum	Plasma	Serum
Range	3.3–69.4	4.0–58.1	76.9–769	33.8–900
Mean	25.4	28.7	292.2	342.1
Median	20.6	19.5	244.0	286.0

Figure Legends

FIG. **1**. Foetal DNA in maternal serum from women carrying aneuploid and normal foetuses. The control and aneuploid groups are as indicated on the x-axis. The foetal SRY DNA concentrations expressed in copies/ml are plotted on the y-axis.

FIG. **2**. Foetal DNA in maternal serum in pre-eclamptic and non-pre-eclamptic pregnancies. The pre-eclamptic and control groups are as indicated on the x-axis. The foetal SRY DNA concentrations expressed in copies/ml are plotted on the y-axis.

FIGS. **3**A and **3**B. Real time quantitative PCR. A, Amplification plots obtained using real time quantitative PCR for the SRY gene. Each plot corresponds to a particular input target quantity marked by a corresponding symbol. The x-axis denotes the cycle number of a quantitative PCR reaction. The y-axis denotes the ΔRn which is the fluorescence intensity over the background (Heid et al. 1996). B, Plot of the threshold cycle (C_T) against the input target quantity (common log scale). The correlation coefficient is 0.986.

FIGS. **4***a*–**4***l*. Sequential study of 12 women bearing male fetuses who conceived by in vitro fertilization. Each case is denoted by a unique recruitment case number. The x-axis denotes the gestation at which the serum sample was obtained. A gestation age of zero denotes the pre-conception sample. The y-axis denotes the concentration of foetal SRY in maternal serum expressed in copies/ml. The scale has been optimized for the concentration range for each case.

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What is claimed is:

1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises

amplifying a paternally inherited nucleic acid from the serum or plasma sample and

detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.

2. The method according to claim **1**, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.

3. The method according to claim **2**, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification.

4. The method according to claim **1**, wherein the foetal nucleic acid is detected by means of a sequence specific probe.

5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.

6. The method according to claim 5, wherein the Y chromosome sequence is from the DYS14 locus.

7. The method according to claim 5, wherein the Y chromosome sequence is from the SRY gene.

8. The method according to claim 1, wherein the presence of a foetal nucleic acid from a paternally-inherited non-Y chromosome is detected.

9. The method according to claim 8, wherein the non-Y sequence is a blood group antigen gene.

10. The method according to claim 8, wherein the non-Y sequence is a gene which confers a disease phenotype in the foetus.

11. The method according to claim 8, for Rhesus D genotyping a foetus in a Rhesus D negative mother.

12. The method according to claim 5, for determining the sex of the foetus.

13. The method according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.

14. The method according to claim 13, wherein the determination of the concentration of foetal nucleic acid sequence in the maternal serum or plasma is by quantitative PCR.

15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal.

16. The method according to claim 13, wherein the pattern of variation of foetal DNA concentration in the maternal serum or plasma at particular stages of gestation is different from normal.

17. The method according to claim 13, for detection of pre-eclampsia.

18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.

19. The method according to claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.

20. The method according to claim 19, wherein the fractional concentration of foetal DNA is at least about 0.39%.

21. A method of performing a prenatal diagnosis, which method comprises the steps of:

(i) providing a maternal blood sample;

(ii) separating the sample into a cellular and a non-cellular fraction;

(iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim 1;

(iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.

22. The method according to claim 21, wherein the non-cellular fraction as used in step (iii) is a plasma fraction.

23. The method according to claim 21, including performing the further step of allowing clotting in the maternal sample and using the resulting serum in step (iii).

24. A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:

removing all or substantially all nucleated and anucleated cell populations from the blood sample,

amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally inherited fetal nucleic acid.

25. A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises

obtaining a non-cellular fraction of the blood sample

amplifying a paternally inherited nucleic acid from the non-cellular fraction

and performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal nucleic acid.

26. The method according to claim 9, wherein the blood group antigen gene is the rhesus D gene.

27. The method according to claim 10, wherein the gene is the rhesus D gene.

* * * * *